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Research Article

Validated High-Performance Liquid Chromatography Method for Quantitative Determination of Anthracenderivatives in Decoction, Syrup and Water-Alcohol Extract of *Frangula alnus* Mill. Bark

Shmygareva A A[1*], Kurkin V A[2], Sankov A N[1]

[1]*Orenburg State Medical University, Department of Economics of Pharmacy, Pharmaceutical Technology and Pharmacognosy, Russia*
[2] *Samara State Medical University, Department of Pharmacognosy, Botany and Phytotherapy, Russia*

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ABSTRACT
Nowadays, people pay much attention to the quality of herbal medicines. Because of the significant variation in active components in them, the quality control of herbal medicines is a very important issue. High-performance liquid chromatography (HPLC) is widely used in identification and quality control of herbal medicines. A new, simple, sensitive, selective, and precise high-performance liquid chromatography (HPLC) was developed for the determination and identification of frangulin A content in "Frangula syrup", "Frangula decoction" and "solution of aqueous-alcoholic extract from the bark of Frangula alnus" was developed and validated. The stationary phase was inert sil C18 column. The mobile phase consisting of acetonitrile (HPLC Grade) and potassium dihydrogen phosphate buffer (pH 2.5) in a gradient flow was used. The column was equilibrated with the mobile phase (flow rate 1.0 ml/min); the UV detection was set at 420 nm. Various optimizations were performed to examine the frangulin A content in herbal medicines and their preparation including retention time, spike authentic standard, change of wavelength, change of mobile phase composition and blank test. Using HPLC analysis we obtained the results of the quantitative determination of content of frangulin A in decoction, syrup and water-alcohol extract of *Frangula alnus* Mill. bark. The content of frangulin A in decoction amounted to 6,45 µg/20 µl, in syrup amounted to 3,72 µg/20 µl, in water-alcohol extract amounted to 7,9 µl /20 µg .

Keyworlds: High-performance liquid chromatography, HPLC, *Frangula alnus* Mill., bark, 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 *Frangula alnus* Mill. bark for the anthracene derivatives[2]. These approaches to standardization were used to develop a quantitative analytical procedure for total anthracenederivatives in *Frangula alnus* Mill. bark syrup that combined analyses in the order raw material – preparation[3,4,5]. The purpose of the present research - to develop qualitative and quantitative methods of analysis anthracenderivatives in the decoction, syrup and water-alcohol extract of *Frangula alnus* Mill. Bark.

RESULTS AND DISCUSSION

Materials

bark of *Frangula alnus* Mill. (OAO "Krasnogorleksredstva"). 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 bark of *Frangula alnus* Mill. 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 *Frangula alnus* Mill. 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 *Frangula alnus* Mill. 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].

**Author for Correspondence: a.shmygareva@mail.ru*

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
Frangula decoction	3,36	25,36	6,45
Frangula water-alcohol extract	4,11	31,40	7,9
Frangula syrup	1,94	14,08	3,72

Table 2: Metrological characteristics of the methods of quantitative determination of the content of frangulin A in *Frangula alnus* decoction:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,061	0,0011	95	2,23	±0,0025	±4,15

Table 3: Metrological characteristics of the methods of quantitative determination of the content of frangulin A in *Frangula alnus* syrup:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,034	0,0007	95	2,23	±0,0016	±4,78

Table 4: Metrological characteristics of the methods of quantitative determination of the content of frangulin A in water-alcohol extract of *Rhamnus cathartica*:

f	$\overline{X}$	*S*	*P, %*	*t (P,f)*	*ΔX*	*E, %*
10	0,075	0,0013	95	2,23	±0,003	±3,97

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 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 content of frangulin A in percentage (X) calculated by the formula:

$$X = \frac{H * m_0 * V_1 * V_2 * 100}{H_0 * 25 * V * V_0}$$

where H is the height of the peak of the frangulin A on the chromatogram of preparation; H_0 – the peak height of the frangulin A (standard sample); V – the volume of aliquots of preparation, ml; V_o – the volume of solution of frangulin A, ml; V_1 – the volume of solution of preparation, ml; V_2 – the volume of aliquots of solution of the frangulin A, ml; m_0 – mass of the frangulin A (standard sample), g.

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 frangulin A in syrup, decoction and water-alcohol extract of *Frangula alnus* Mill. is varied from 0,034 % to 0,075% respectively.

The metrological characteristics of the developed method of HPLC analysis showed that a single error determination of the content of frangulin A in *Frangula alnus* Mill. 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 the content of frangulin A in *Frangula alnus* 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 the content of frangulin A in water-alcohol extract of *Frangula alnus* with a confidence level of 95% is ± 3,97% (Table. 4).

CONCLUSIONS

Developed methodological approaches to the standardization of decoction, syrup and water-alcohol extract of *Frangula alnus* Mill. bark, consisting in the determination of the content of frangulin A and the using

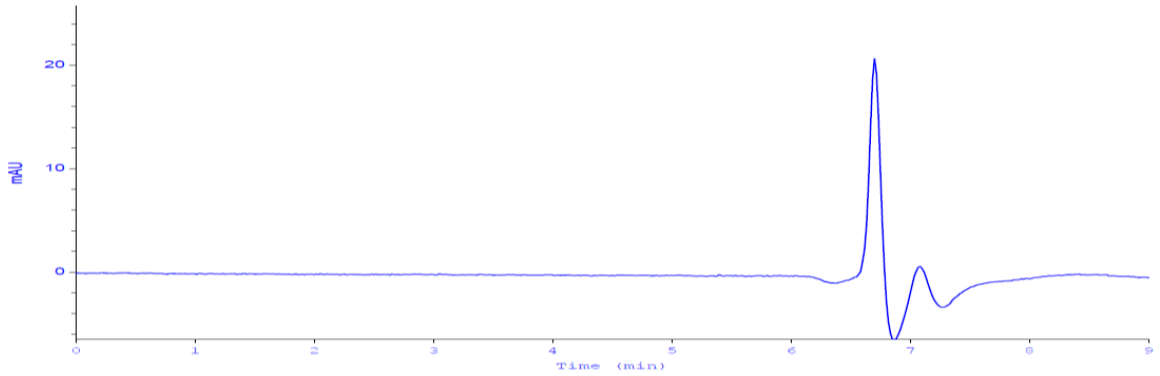

Figure 1: HPLC of Frangula decoction. Solvent system: 20 % buffer B. Absorbance at 420 nm.

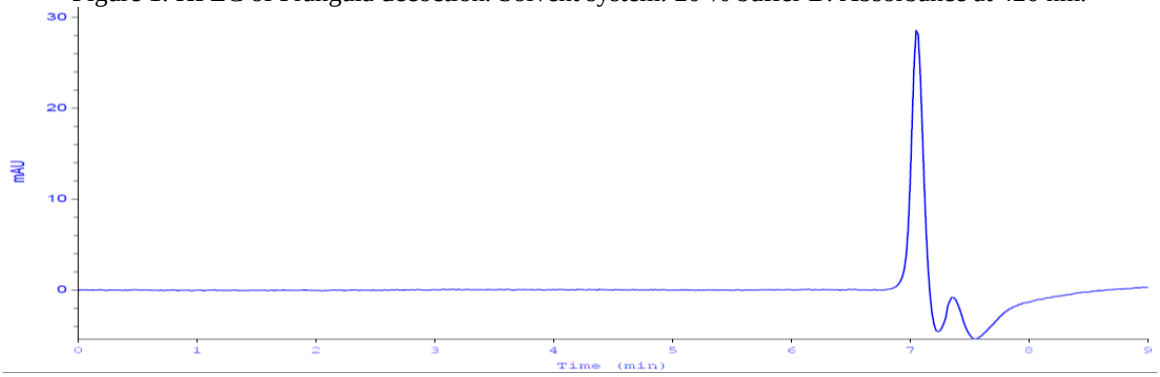

Figure 2: HPLC of Frangula water-alcohol extract. Solvent system: 20 % buffer B. Absorbance at 420 nm.

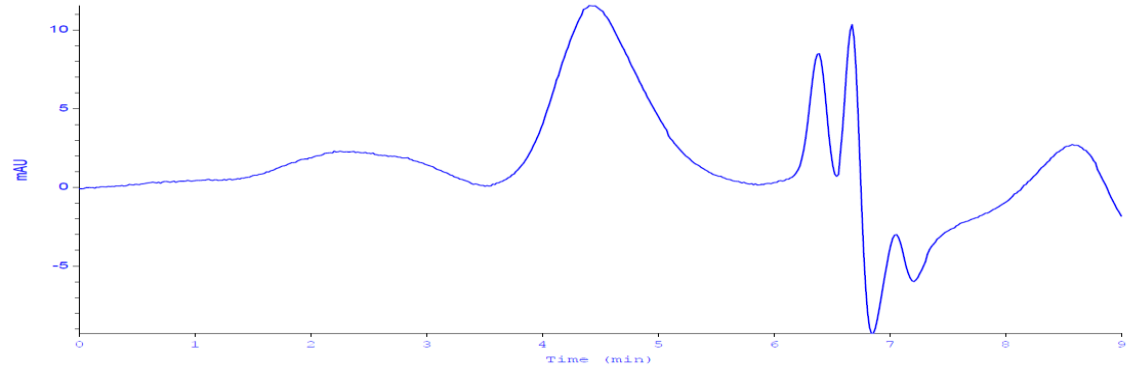

Figure 3: HPLC of Frangula syrup. Solvent system: 20 % buffer B. Absorbance at 420 nm.

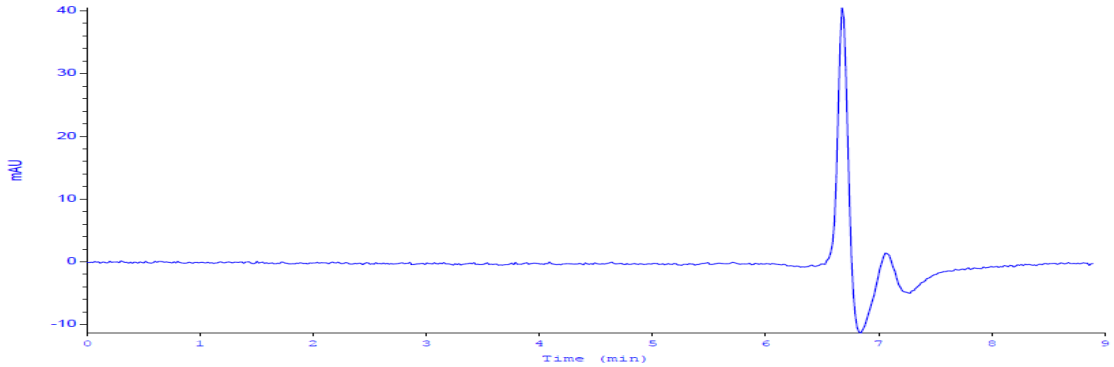

Figure 4: HPLC of frangulin A (standard sample). Solvent system: 20 % buffer B. Absorbance at 420 nm.

of techniques of the analysis of standard sample of Frangulin A. The method of quantitative determination of the content of frangulin A in the anthracenderivative preparations using HPLC at the analytical wavelength 420 nm was carried out. The content of frangulin A in decoction amounted to 6,45 µg/20 µl, in syrup amounted to 3,72 µg/20 µl, in water-alcohol extract amounted to 7,9 µg /20 µl .

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