

UDC 615.07:615.32:582.711.71:54.061

**DEVELOPMENT OF METHOD OF QUALITATIVE
ANALYSIS OF BIRD CHERRY FRUIT FOR
INCLUSION IN THE MONOGRAPH OF STATE
PHARMACOPOEIA OF UKRAINE**

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Introduction

Bird cherry *Padus avium* Mill (synonym *Prunus padus* L.) from family *Rosaceae*, is widespread in Ukraine, especially in forests and forest-steppe areas, in the Carpathians it rises to the upper bound of the forest [1]. Bird cherry fruits have long been used in medicine and is a valuable medicinal raw materials. They stated to possess astringent, anti-inflammatory, phytoncidal properties. As a decoction they have been used for enteritis, dyspepsia of different etiology; externally – for chronic colpitis [2-4]. Bird cherry fruits are included in the State Pharmacopoeia of several countries: The USSR Pharmacopoeia IX ed., The State Pharmacopoeia of the Russian Federation, The State Pharmacopoeia of the Republic of Belarus [5-7]. In Ukraine there are no contemporary normative documents for this medicinal plant material, therefore it is the actual to develop projects of the national monographs "bird cherry fruit dry" and "bird cherry fruit fresh" to be included in the State Pharmacopoeia of Ukraine. Harmonization of requirements for quality and safety of medicines is the main focus in the development of standards and creating pharmacopoeia monographs in the world [8]. According to European Pharmacopoeia recommendation method of thin-layer chromatography (TLC) is prescribed only for the identification of the herbal drug [9]. The principles of thin-layer chromatography and application of the technique in

pharmaceutical analysis are described in the State Pharmacopoeia of Ukraine [10]. As it is effective and easy to perform, and the equipment required is inexpensive, the technique is frequently used for evaluating medicinal plant materials and their preparations [11]. TLC is used under Identification, even if other chromatographic methods, such as gas chromatography (GC) and liquid chromatography (LC) are subsequently used in the monograph. In this context the TLC is aimed at elucidating the chromatogram of the drug with respect to selected reference compounds that are described for inclusion as reagents [9].

Aim of this study was to develop methods of qualitative analysis of bird cherry fruits for a monograph in the State Pharmacopoeia of Ukraine (SPU).

Materials and Methods

The object of our study was dried bird cherry fruits (7 samples) and fresh bird cherry fruits (6 samples) harvested in 2013-2015 in Kharkiv, Poltava, Luhansk, Sumy, Lviv, Mykolaiv regions and the city Mariupol. Samples were registered in the department of SPU State Enterprise "Pharmacopoeia center".

In accordance with the Ph. Eur. and SPU requirements in "identification C" determination was performed by TLC. TLC was performed on glass-backed silica gel F₂₅₄Merck plates, size 20x10 cm. To prepare the test solution dry raw material was chopped to powder, and fresh raw material was crushed to mash. Test solutions from samples of dried raw material were prepared by the following procedure.

To 1 g of dried chopped bird cherry fruits (exact sample), sifted through a sieve № 1.25, 25 mL of 1% solution of hydrochloric acid in 95% ethanol was added and treated with ultrasound for 60 minutes at 50° C, then filtered. The filtrate was evaporated to half volume. As mobile phase solvent mixture ethyl acetate - anhydrous acetic acid - formic acid - water ratio (100: 10: 10: 25) was selected.

Data about the series, registration number, and sample weight for the preparation of the test solution are given in Table 1.

Table 1. Series, registration number, mass of sample of "bird cherry fruit dry" for the preparation of the test solution

№	Series	Location of harvesting	Registration number *	Mass of sample, g
1	0760	Raw materials purchased, the manufacturer - the company "Sumy fitofarmatsiya"	RS 432	1,00295
2	020815	Botanical Garden of NUPh, city Kharkiv	RS 436	1,00739
3	110815	Lviv region, forest	RS 437	1,00972
4	250815	City Mariupol, croft	RS 438	1,00432
5	310815	Poltava region, Mirgorod, forest	RS 439	1,00430
6	10092015	Mykolaiv region, forest	RS 447	1,00385
7	082010	Lugansk region, croft	RS 457	1,00499

* Registration number in State Enterprise "Pharmacopoeia center"

For preparation the reference solution 1 mg chrysanthem in (Cyanidin-3-glucoside chloride, > 98%, Chengdu Dioputify Phytochemicals Ltd, Lot 15041502) was dissolved in 10 mL of 1% solution of hydrochloric acid in 95% ethanol.

Test solutions and the reference solutions were placed onto the chromatographic plate 5 mcL each. After the plate was dried in air for 5-10 minutes and placed into the chamber.

When the chromatogram developed, the plate was taken out and allowed the solvent to evaporate at room temperature, then observed the spots in daylight.

To determine the presence of anthocyanins in fresh bird cherry fruits test solutions of the samples were prepared using the following method.

To 2 g of fresh bird cherry fruits (exact sample), crushed to mash (free of seeds), 10 mL of methanol was added and treated with ultrasound for 15 minutes, then filtered. As mobile phase solvent mixture formic acid anhydrous-water-butanol ratio (16:19:65) was selected.

For preparation the first reference solution 2 mg chrysanthem in was dissolved in 5 mL of methanol. For preparation the second reference solution Pharmacopoeial Reference Standard SPU of bird cherry extract was dissolved in 0.2 mL of methanol with ultrasound bath.

Data about the series, registration number, and sample weight of fresh bird cherry fruits for the preparation of the test solution are given in Table 2.

Table 2. Series, registration number, mass of sample of "bird cherry fruit fresh" for the preparation of the test solution

№	Series	Location of harvesting	Registration number*	Mass of sample, g
1	5070915	Botanical garden of V. N. Karazin Kharkiv National University, city Kharkiv	RS 440	2,08307
2	4070915	Botanical Garden of National University of Pharmacy, city Kharkiv	RS 441	2,09104 г
3	2070915	Lviv region, forest	RS 442	2,16275
4	3070915	City Mariupol, croft	RS 443	2,04312
5	6070915	Mykolaiv region, forest	RS 445	2,03796
6	7070915	Lugansk region, croft	RS 446	2,06129

* Registration number in State Enterprise "Pharmacopeia center"

Test solutions and the reference solutions were placed onto the chromatographic plate 5 mcL each. After the plate was dried in air for 5-10 minutes and placed into the chamber.

When the chromatogram developed, the plate was taken out and allowed the solvent to evaporate at room temperature, then observed the spots in daylight.

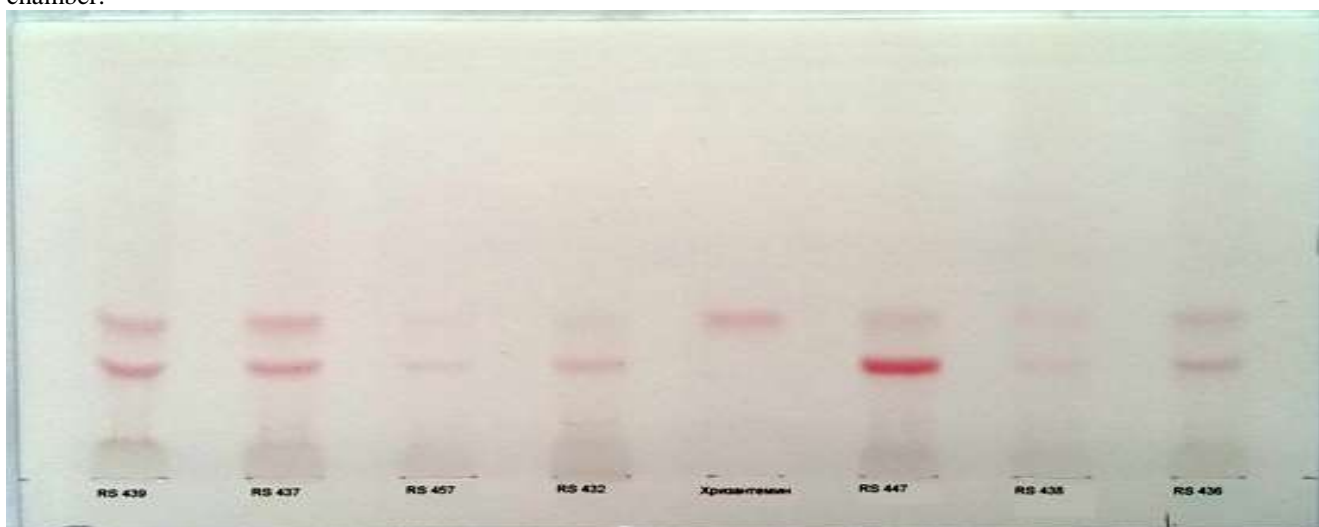


Figure 1. TLC of anthocyanins of dried bird cherry fruit, where RS 432, RS 436, RS 437, RS 438, RS 439, RS 447, RS 457 – samples

Results and discussion The chromatographic analysis of samples of dried bird cherry fruit showed two dominant

spots, one of which coincided with chrysanthemine (Figure 1).

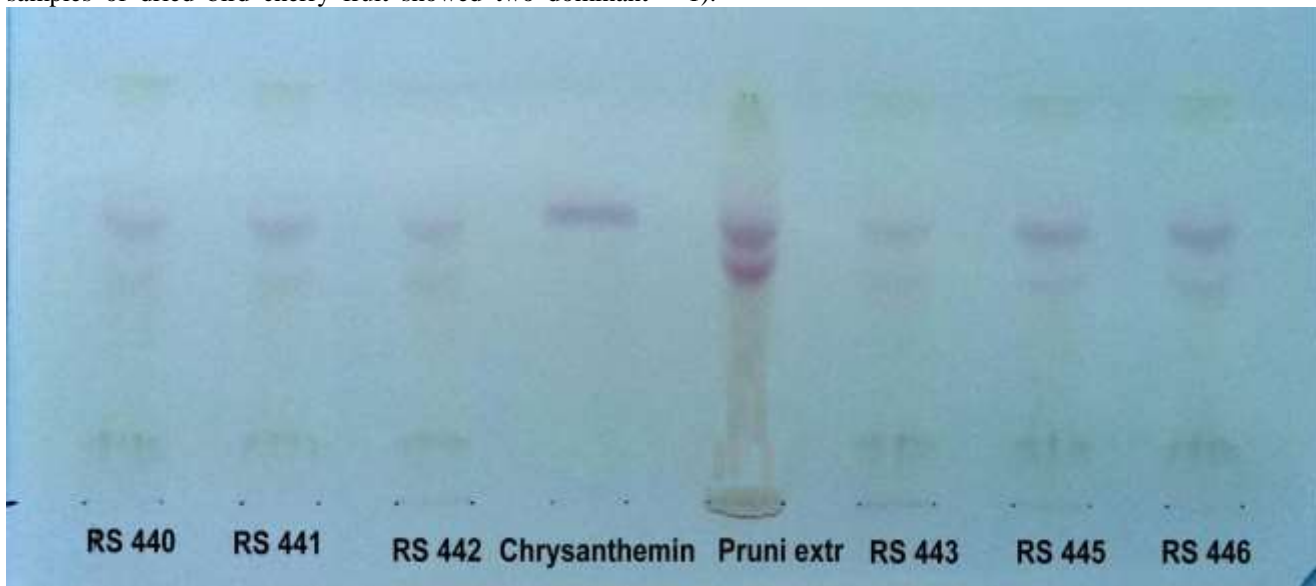


Figure 2. TLC of anthocyanins of fresh bird cherry fruit, where RS 440, RS 441, RS 442, RS 443, RS 445, RS 446 – samples

In the chromatogram it was observed for all dry bird cherry fruits samples two pink-red color zones, one of which coincided for Rf and color of chrysanthemine, and the other one was slightly lower.

Therefore the sequence of zones for presentation to the SPU monograph project "Bird cherry fruits dry" should be as follows: on examination in the daylight two pink-red zones of the test solution can be seen, the top zone must be located exactly opposite chrysanthemine reference solution zone.

The results of chromatographic analysis of fresh bird cherry fruit are presented on Figure 2.

The chromatographic analysis of samples of fresh fruits showed two dominant pinkish-violet spots, one of which had color and Rf coincided with first reference solution chrysanthemine and also both zones coincided with two zones of the second reference solution Pharmacopoeial Reference Standard SPU of bird cherry extract.

Conclusion

1. Analysis of bird cherry fruits dried and fresh harvested in Ukraine was carried out by TLC.
2. It was confirmed the opportunity to include in the national monographs "Bird cherry fruits fresh" and "Bird cherry fruits dry" in section "Identification C" analysis of anthocyanin by TLC in chosen systems and methods.

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Therefore the sequence of zones for presentation to the SPU monograph project "Bird cherry fruits fresh" should be as follows: on examination in the daylight two pink-violet zones of the test solution can be seen, the top zone must be located exactly opposite chrysanthemine reference solution zone or both of them should have color and Rf coincided with two pinkish-violet zones of reference solution Pharmacopoeial Reference Standard SPU of bird cherry extract.

Hence, we have developed a method of identification of fresh and dry bird cherry fruits by TLC. In accordance with the requirements of Ph. Eur. identification by TLC is obligatory. Division of anthocyanins of plant raw materials was observed sufficiently in our chosen solvent systems performed by these methods of analysis that allows to recommend these systems and methods of analysis for inclusion in the relevant sections of monographs for bird cherry fruit, dried and fresh for SPU.

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Introduction. Bird cherry *Padus avium* Mill, *Rosaceae*, is widespread in Ukraine, especially in forests and forest-steppe areas. Bird cherry fruits have long been used in medicine and is a valuable medicinal raw materials. They stated to possess astringent, anti-inflammatory, phytoncidal properties. Bird cherry fruits are included in the USSR Pharmacopoeia IX ed., The State Pharmacopoeia of the Russian Federation, The State Pharmacopoeia of Republic of Belarus. In Ukraine there are no contemporary normative documents for this medicinal plant material, therefore it is the actual to develop projects in the national monographs "dry bird cherry fruit" and "fresh bird cherry fruit" to be included in the State Pharmacopoeia of Ukraine. According to European Pharmacopoeia recommendation method of thin-layer chromatography (TLC) is prescribed only for the identification of the herbal drug. The principles of thin-layer chromatography and application of the technique in pharmaceutical analysis are described in State Pharmacopoeia of Ukraine. As it is effective and easy to perform, and the equipment required is inexpensive, the technique is frequently used for evaluating medicinal plant materials and their preparations. The TLC is aimed at elucidating the chromatogram of the drug with respect to selected reference compounds that are described for

inclusion as reagents. Aim of this study was to develop methods of qualitative analysis of bird cherry fruits for a monograph in the State Pharmacopoeia of Ukraine (SPU). **Materials and Methods.** The object of our study was dried bird cherry fruits (7 samples) and fresh bird cherry fruits (7 samples) harvested in 2013-2015 in Kharkiv, Poltava, Luhansk, Sumy, Lviv, Mykolaiv regions and the city Mariupol. Samples were registered in the department of SPU State Enterprise "Pharmacopoeia center". In accordance with the Ph. Eur. and SPU requirements in "identification C" determination was performed by TLC. TLC was performed on glass-backed silica gel F₂₅₄Merck plates, size 20x10 cm. Test solutions from samples of dried raw material were prepared by extraction of 1% solution of hydrochloric acid in 95% ethanol with ultrasound for 60 minutes at 50° C. As mobile phase solvent mixture ethyl acetate - anhydrous acetic acid - formic acid - water ratio (100: 10: 10: 25) was selected. For preparation the reference solution 1 mg chrysanthem in was dissolved in 10 mL of 1% solution of hydrochloric acid in 95% ethanol. Fresh bird cherry fruits test solutions of the samples were crushed to mash (free of seeds), 10 mL of methanol was added and treated with ultrasound for 15 minutes. As mobile phase solvent mixture formic acid anhydrous-water-butanol ratio (16:19:65) was selected. Two reference solutions were used. First was prepared from 2 mg chrysanthemum dissolved in 5 mL of methanol and second was Pharmacopoeial Reference Standard SPU of bird cherry extract dissolved in 0.2 mL of methanol with ultrasound bath. Test solutions and the reference solutions were placed onto the chromatographic plates and placed into the chamber. When the chromatograms developed, the plates were taken out, then observed the spots in daylight. **Results and discussion.** In the chromatogram it was observed for all dry bird cherry fruits samples two pink-red color zones, one of which coincided for R_f and color of chrysanthem in, and the other one was slightly lower. The chromatogram for all samples of fresh raw material showed two pinkish-violet zones, one of which had R_f and color corresponded chrysanthem in, and the second one was slightly lower. Compared with bird cherry extract, it was observed two pinkish-violet zones, coincided with R_f and color of bird cherry (SPU) extract zones. In accordance with the requirements of Ph. Eur. identification by TLC is obligatory. Division of anthocyanins of plant raw materials was observed sufficiently in our chosen solvent systems and methods of analysis that allows to recommend these systems and methods of analysis for inclusion in the relevant sections of monographs for bird cherry fruit, dried and fresh for SPU. **Conclusion.** It was confirmed the opportunity to include in the national monographs "Bird cherry fruits fresh" and "Bird cherry fruits dry" in section "Identification C" analysis of anthocyanin by TLC in chosen systems and methods.

Key words: bird cherry, TLC, State Pharmacopoeia of Ukraine