Quantification of scoparone in wormwood grass using thin-layer chromatography

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Abstract. The method to identify and quantify scoparone content in wormwood grass using thin-layer chromatography was developed and validated. For the experiment, the above-ground part (grass) of the panicle wormwood in the flowering phase collected on the territory of the collection nursery of the Nikitsky Botanical Gardens in 2019 was used. An alcohol extraction containing coumarin scoparone – phytotoxic and pharmacologically active compound – was prepared via bismaceration. Sorbfil plates (PTCH-P-A-UV), mobile phase were used for chromatography: petroleum ether – ethyl acetate – ethanol (4:1:1). The scanning zones were identified in ultraviolet light using an UV lamp (254 nm). Digital analysis of chromatograms was carried out using Sorbfil Videodensitometer software. The technique is validated according to the following characteristics: sensitivity, specificity, linearity, limit of quantitative determination, correctness, intra-laboratory precision. The content of scoparone in the wormwood grass is 0.35±0.03 %.

1. Introduction

Wormwood (Artemisia scoparia Waldst. et Kit. of Asteraceae family) is an annual, less often biennial herbaceous plant. In Russia, it is distributed in the central and southern regions of the European part, in the Crimea, in the Caucasus, in the south of Siberia and the Far East. It is clear that the significant area distribution of this species is largely caused not only by its adaptation characteristics, but also by the characteristics of the profile of secondary metabolites. Essential oil contains more than 80 % of polyacetylene compounds, including capillin, capillen, capillol and others, possessing powerful antimicrobial properties [1, 2]. Another group of compounds – flavones (arcapillin, capillarizin) are antioxidants [3]. Equally important is the group of phenolic lactones – these are such coumarins as scoparone, scopoletin, umbelliferone. For some of these compounds, phytotoxic activity is described, which determines the ability of the plant to compete in phytocenoses [3–6].

Plant grass and roots are used for medicinal purposes. In folk medicine, grass is used to treat menstrual disorders and as a helminthagogue agent. The plant is popular in Chinese, Tibetan and Indian medicine. Artemisiae scopariae herba represents dried shoots (6–10 cm); as a medicinal raw material it is included in the Pharmacopoeia of China and is used mainly as a bile, anti-inflammatory and diuretic agent in the treatment of epidemic hepatitis [7].
Chinese scientists conducted a study of the chemical composition of methanol extraction from wormwood grass by HPLC-fingerprint analysis and thin-layer chromatography (TLC-fingerprint analysis). The following solvent system was used for thin-layer chromatography: toluene-ethyl acetate-formic acid-water (5:100:10:10). Detection on plates was carried out in ultraviolet at a wavelength of 365 nm. Umbelliferone and scopoletin were found from a group of coumarins. Data on the content of wormwood scoparone in grass and its amount are not given. However, it is known that scoparone is one of the main biologically active substances of wormwood grass [8].

Scoparone (6,7-dimethoxy coumarin) is a biologically active substance and has hepatoprotective activity (protects liver cells), as well as a stimulating effect on bile secretion and gallbladder contraction [5, 6].

![Figure 1. Structural formula of scoparone](image-url)

The Nikitsky Botanical Gardens conducts crop research to study the samples of panicle wormwood, which were introduced from Azerbaijan, Georgia and some regions of Russia in order to identify the most promising forms for industrial cultivation in the southern Crimea. In addition to essential oil, a group of coumarins and, in particular, scoparone contained in the above-ground part of the panicle wormwood is particularly interesting [9].

2. Purpose of the study
The purpose of the study was to develop and validate a method for quantifying scoparone in the grass of cultured samples. To do this, the most available method of thin-layer chromatography (TLC) was used. Modern computer technologies make it possible to quantitatively analyze TLC with great sensitivity, efficiency and with an error that is quite permissible for the analysis of plant extracts.

Quantitative processing of chromatograms is carried out using densitometers in computer programs. The principle of video densitometry is based on the introduction of a chromatogram image into a computer with subsequent comparison of the color intensity of the spots (scanning zones) of standard and studied compounds [10].

3. Materials and methods
The object of the study included samples of the above-ground part (grass) of the panicle wormwood collected in the flowering phase on the territory of the collection nursery of the Nikitsky Botanical Gardens in 2019. The extract was obtained by bismaceration using a five-fold amount of an extractant. 70% ethyl alcohol was used as the extractant.

1. Preparation of a standard sample (SS) of scoparone solution
A suspension weighing 0.025 g (exact suspension) of SS scoparone (Sigma-Aldrich, 6,7-dimethoxycoumarin) was placed in a measuring flask with a capacity of 25 ml. About 10 ml of ethyl alcohol (95%) was added. The flask was heated in a water bath to dissolve the substance, and then cooled to room temperature, and the volume of the solution was adjusted with ethyl alcohol to a mark, the solution was stirred. The concentration of the resulting solution was 1 μg/μl.

2. Preparation of a chromatogram
A 15 cm long chromatographic plate was started with 1, 2, 3, 4, 5 μl SS scoparone samples containing 1, 2, 3, 4, 5 μg of the substance in the stain and 10 μl of alcohol extract. The solution was applied with GC MLT-10 microsyringe.

3. Plate chromatography
Chromatography was performed using Sorbfil PTCH-P-A-UV plates. Eluting system: petroleum ether-ethyl acetate-ethanol (4:1:1). Spot detection was performed in ultraviolet light with a 254 nm UV lamp.

The plate was placed in a chromatography chamber pre-saturated with solvent vapor. The solvent front path was about 9 cm. After chromatography, the plate was removed from the chamber, dried in a hood until the solvent vapor odor was completely removed.

For digital processing of chromatograms, Sorbfil Videodensitometer computer program (Krasnodar) was used. Quantitative determination was carried out by absolute calibration (external standard), according to the calibration dependence graph “mass of substance – peak area”. Statistical processing of the results was carried out in accordance with the requirements of the GPS “Statistical processing of the results of a chemical experiment and biological tests” [11, 12].

Sensitivity, specificity, linearity, limit of quantitative determination, correctness, intralaboratory precision were determined as the main characteristics assessing the validity of the method.

4. Results and discussion

On the surface of the plate on tracks with SS scoparones and on tracks with the extraction, spots of bright purple glow with Rf 0.57±0.02 appeared.

The plate was scanned, the image was processed with a computer program and a digital chromatogram was obtained (Fig. 2). On the digital chromatogram, the peak of SS scoparone coincides with the peak on the extraction tracks.

**Figure 2.** Chromatogram of extraction from wormwood (track 2) and SS scoparone (track 1)

4.1 Linearity

To confirm the linearity of the calibration diagram, the results of determining the areas of SS scoparone spots were processed by the least squares method, the significance of the intercept term, the regression equation, and the correlation coefficient were determined.

The results of statistical processing of experimental data confirm that the procedure for quantifying scoparone in the mass range from 1 to 5 μg is linear. An intercept term does not matter. The regression equation looks as follows: \( S = 5.9 \times 10^4 \) (Fig. 3). The correlation coefficient \( r = 0.995 \).
4.2 Sensitivity
According to the calibration diagram, the sensitivity of the method was determined: the detection limit (DL) and the quantitative detection limit (QDL) were calculated. The detection limit was calculated according to the formula: \( DL = 3.3 \times S/b \), where: \( S \) – standard deviation of a signal; \( b \) – angular coefficient of the calibration diagram. \( DL = 1.47 \mu g \). The quantitative detection limit (QDL) was calculated according to the formula: \( QDL = 10 \times S/b \), \( QDL = 4.46 \mu g \).

The correctness of the procedure was determined by the “introduced-found” method at five levels of SS scoparone concentrations with two results at each level. The results are as follows: \( X_{av} = 97.3 \% \); \( \Delta X = 7.9 \% \); \( RSD \% = 11 \% \); \( E = 8.2 \% \).

4.3 Intralaboratory precision
To obtain statistically correct quantification data, the test was performed on several plates using at least two tracks of the control sample. The results were processed using the Student mathematical statistics. The results are reported in terms of air-dry raw materials (Table 1).

Table 2. Scoparone content in wormwood grass

| Scoparone content, g/100 g air-dry raw material | Metrological characteristics |
|------------------------------------------------|-----------------------------|
| 0.31; 0.31; 0.33; 0.33; 0.35; 0.35; 0.37; 0.38; 0.40; 0.41 | \( X_{av} = 0.35 \% \)  
\( \Delta X = 0.03 \% \)  
\( E = 7.14 \% \) |

The literature suggests using different methods of analysis to determine coumarin scoparone (6,7-dimethoxycoumarin) in plant raw materials. These include thin-layer chromatography, UV spectrophotometry, high performance liquid chromatography, and some others [5–8]. Some of them (TLC, UV spectrophotometry) are quite simple and cost-efficient, however, they are either not validated or give a large determination error. Others (LC-UV) require expensive equipment and consumables. The obtained experimental data show that TLC combined with video sensitometry using a simple technique and low costs for consumables provides for valid results with a relatively small error.

5. Conclusion
The possibility of quantitative determination of coumarin scoparone in alcohol extraction from vegetal raw materials by thin-layer chromatography is shown. A sensitive, highly efficient technique that does not require expensive equipment was developed and validated.
Under the conditions of the experiment, it was determined that the content of scoparone in the wormwood grass cultivated on the southern coast of Crimea is 0.35±0.03% in terms of air-dry raw materials.

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