Development and validation of an analytical method for assessing the authenticity of Vaccinium myrtillus shoots L.

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Development and validation of analytical method for verification of identity of shoots of Vaccinium myrtillus L.

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SUMMARY

A method has been developed for determining the authenticity of common blueberry shoots by chromatography in a thin layer of sorbent. The optimal conditions for chromatographic analysis have been determined. The method was validated according to the following parameters: specificity, precision and robustness. It was found that all the validation characteristics under study are within the acceptance criteria. The results of validation made it possible to include the developed methodology in the draft of the pharmacopoeial monograph “Common bilberry shoots - whole, crushed and Angro powder,” adopted for consideration by the FGBU “NTs ESMP” of the Ministry of Health of Russia (input No. 6473 of 23.04.13).

Key words: validation, chromatography in a thin layer of sorbent, specificity, precision, robustness, bilberry shoots, flavonoids.

RESUME

A method was developed for verification of identity of bilberry shoots by chromatography in a thin layer of sorbent. The optimal conditions for the chromatographic analysis were stated. The method was validated to ensure specificity, precision and robustness. Statistical calculations proved the validation parameters to be within the boundaries of acceptance criteria. The results of the validation allowed to include the method into pharmacopoeia monograph “Bilberry shoots: whole, crushed and powdered”, adopted for approval by FSBI Scientific Center for Expertise of Medical Products of Russian Ministry of Health (Ref. No. 6473, April 23, 2013).

Keywords: validation, chromatography in a thin layer of sorbent, specificity, precision, robustness, bilberry shoots, flavonoids.

Due to the compositional diversity of its chemical composition, the standardization of medicinal plant materials is a very difficult analytical problem. It is practically impossible to carry out the identification of raw materials based on the data of the primary phytochemical stage of determining its authenticity - qualitative reactions. Qualitative reactions only make it possible to ascertain the presence of a group or groups of biologically active substances in the raw material. The most specific in this respect are chromatographic methods of analysis.

In FS-42-2948-93, regulating the quality of shoots Vassinium myrtillus L., the authenticity of raw materials is determined by only one qualitative reaction with iron-ammonium alum for tannins. The purpose of this work is to develop and validate a chromatography technique in a thin layer of sorbent to determine the authenticity of shoots.V. myrtillus L.

MATERIALS AND RESEARCH METHODS

The objects of study were shoots V. myrtillus L., collected from the range of the species. Chromatography in a thin layer of sorbent was performed on Sorbfil PTSKh-P-V plates (Russia) with a size of 100 x 100 mm and TLC Silica gel 60 F254 Alluminium sheets (Merck, Germany) with a size of 200 x 200 mm. Samples were applied to the plates using a Linomat V semi-automatic applicator (Camag, Switzerland). We used a standard hyperoside sample (Sigma Aldrich, Cat. No 00180585) [1].

The method was validated in accordance with the established requirements. In the course of the work, such validation characteristics as specificity, precision and robustness were considered in accordance with the rules and recommendations [2, 3].

RESULTS AND ITS DISCUSSION

When developing the TLC method, the issues of sample preparation for analysis were studied - the type of extractant, extraction time, extraction conditions, as well as the composition of the chromatographic system, the type of plates, the detection scheme and reagent, the amount of sample applied to the plate, preparation of the chamber (saturation time) and plates for chromatography. , the path length of the mobile phase.

It was experimentally found that it is advisable to use the extract obtained with 70% ethyl alcohol as the test solution. Meanwhile, the analysis of the chromatographic profile of the crude extract showed that the adsorption zones corresponding to flavonoids are not clearly divided, a number of zones overlap, which greatly complicates the determination of their chromatographic characteristics. In this regard, the prospects of using the purification stage by the selective extraction method in the analysis were studied. For this, the alcoholic extract was evaporated under vacuum to an aqueous residue, the precipitate of lipophilic substances was filtered off, then the thickened aqueous extract was washed several times with chloroform until the latter was discolored to remove chlorophyll and resin residues. To obtain a polyphenol fraction, the aqueous residue was further subjected to extraction with ethyl acetate. As a result of the purification, chromatograms with clear and compact adsorption zones were obtained, representing a chromatographic polyphenol image specific forV. myrtillus L.

When choosing the optimal volume of the applied sample, the features of the morphology and chemical composition of the raw material were taken into account. V. myrtillus L. First, shoots are a heterogeneous raw material, and the content of stem parts in it, according to the data of commodity analysis, can vary from 40% to 70%. It was found that the polyphenols of leaves and stems differ both in component composition and in quantitative content. Secondly, the polyphenolic composition of the raw material of the studied species is characterized by a certain natural variability: the maximum and minimum content of flavonoids and tannins in the samples differs 3 times and 1.8 times, respectively [4]. Therefore, to obtain objective chromatographic results, it is proposed to apply two volumes of the analyzed sample to the plate - 5 µl and 10 µl. This approach is a non-standard solution for a similar kind of techniques, but is justified for raw materials.V. myrtillus L.

The choice of the remaining chromatographic parameters was carried out according to a number of criteria - a clear separation and compactness of adsorption zones, as well as a rational arrangement of zones on a chromatographic plate. The selected values of the chromatographic parameters are given in table. 1. The validation assessment of the developed chromatographic technique was carried out according to the following characteristics: specificity, precision, and robustness.

Table 1

Chromatographic conditions

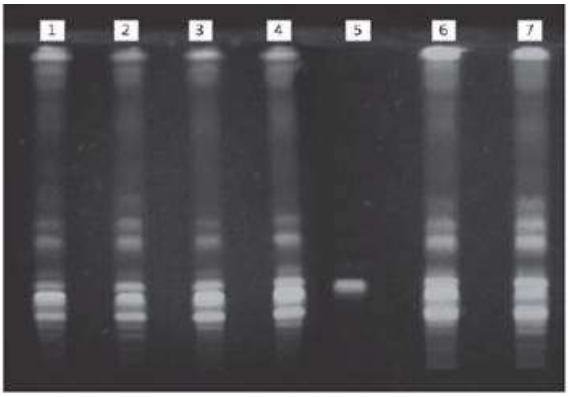
Параметр	Значение
Пластинка	«TLC Silica gel 60 F254» Alluminium sheets (Merck, Германия) размером 200 х 200 мм
Подвижная фаза	этилацетат-хлороформ-спирт метиловый-вода- кислота муравьиная (20:1:1:1:1)
Объем наносимой пробы	5 мкл и 10 мкл
Стандартный образец	гиперозид (Sigma Aldrich, Cat. No 00180585)
Пробег подвижной фазы	15 см
Реагент детектирования	4 % раствор алюминия хлорида в спирте этиловом 95 %

The specificity of the developed technique was confirmed by visual comparison chromatographic profiles of different series V. myrtillus L., collected from the range of the species in different periods of the growing season of the plant, as well as by visual comparison of the adsorption zone in the tracks of the test samples by color and distance from the start line to the adsorption zone in the track of the hyperoside standard sample solution.

As seen in Fig. 1, chromatograms of the test samplesV. myrtillus L. the number, color, intensity and positions of the adsorption zones coincide with each other, and in the tracks of the test samples, a clear definition of the adsorption zone is provided, corresponding in color and distance from the start line to the adsorption zone of the standard hyperoside sample.

To determine the repeatability of R valuesf adsorption zones in the tracks of the test samples, corresponding to the adsorption zone of the standard hyperoside sample, 3 series were used V. myrtillus L. Each sample was applied to a chromatographic plate in triplicate. The studies were carried out over a short time interval, observing synchronous conditions - the analysis was performed by the same analyst using chromatographic plates and reagents of the same series.

When conducting studies of intermediate and interlaboratory precision, all possible required laboratory circumstances were taken into account, which determine intra- and interlaboratory variability: employees, time drift, chromatographic plates of different series, reagents and solvents of different series, etc. To assess these validation parameters, the convergence data presented in Table 1 were used. 1 and a dataset from a second analyst performing the procedure on a different day and data from a second laboratory.



Rice. 1. Chromatograms of samplesVassinium myrtillus L. (tracks 1-4, 6-7), collected over the range of the species and chromatogram of a standard hyperoside sample (track 5).

The acceptance criterion is the variability of Rf values, which for three replicates on one plate should not exceed 0.01, for repeatability - 0.02, for intermediate precision - 0.05 and 0.07 for reproducibility [5, 6].

As you can see from the table. 2 and 3, the obtained research results do not exceed the established acceptance criteria, which indicates the precision of the developed technique under conditions of repeatability, intermediate precision and reproducibility. The robustness of the technique was carried out at the stage of developing the technique. The following were worked out: extractant, extraction time, composition of the mobile phase, amount of applied sample, types of chromatographic plates, etc. The results obtained were reflected in the validated method.

table 2

The results of evaluating the repeatability of the developed technique

Rf зон адсорбции в испытуемых образцах, соответствующих зоне адсорбции стандартного образца гиперозида								
Пластинка 1			Пластинка 2			Пластинка 3		
0,25	0,25	0,25	0,25	0,25	0,25	0,24	0,24	0,24
ΔRf = 0,01								

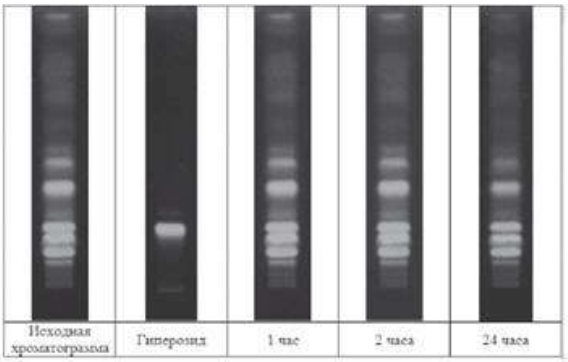
Table 3

Results of evaluating intermediate precision and reproducibility of the developed technique

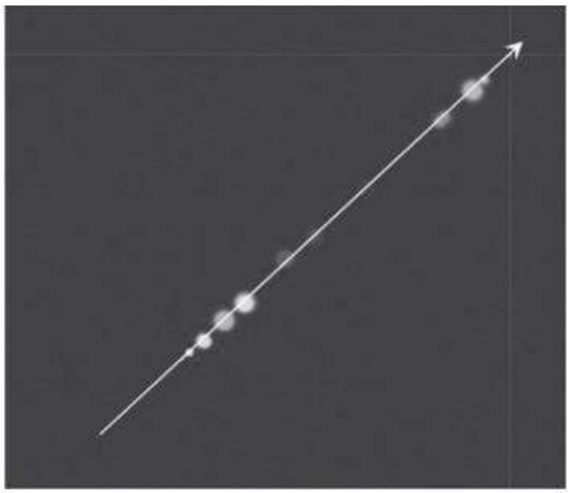
Номер пробы	Промежуточная (внутрилабораторная) прецизионность		Воспроизводимость (межлабораторная прецизионность)
	Аналитик 1	Аналитик 2	
	Rf зон адсорбции в испытуемых образцах, соответствующих зоне адсорбции стандартного образца гиперозида		
1	0,25	0,26	0,27
2	0,25	0,26	0,27
3	0,25	0,26	0,27
4	0,25	0,25	0,26
5	0,25	0,25	0,26
6	0,25	0,25	0,26
7	0,24	0,26	0,27
8	0,24	0,26	0,27
9	0,24	0,26	0,27
ΔRf	0,02		
	0,03		

At the validation stage, studies were also carried out on the stability of the test samples before chromatography and during chromatography. To assess the stability of the test samples before chromatography, the ethyl acetate fraction was applied to the chromatographic plate immediately after preparation, and then after 1 hour, 2 hours and 24 hours. Evaluation of the stability of the test samples during chromatography was confirmed using two-dimensional thin layer chromatography.

In fig. 2 shows the assessment of the stability of the test samples before chromatography, where it is seen that the adsorption zones coincide in number, distance from the starting line, color, intensity and shape. Satisfactory results were also obtained for the stability of the tested samples during chromatography. Photo 3 shows that the adsorption zones in the chromatogram lie on one straight line between the point of application of the intersection of the fronts of the mobile phases. Thus, the developed technique is robust.



Rice. 2. Evaluation of the stability of the test samples before chromatography.



Rice. 3. Evaluation of the stability of the sample during chromatography.

CONCLUSIONS

1. Developed and validated a method for chromatographic determination of the authenticity of shoots V. myrtillus L.

2. The results obtained are included in the draft of the pharmacopoeial monograph "Common bilberry shoots - whole, crushed and "Angro" powder, approved for consideration by the Federal State Budgetary Institution "NTs ESMP" of the Ministry of Health of Russia (input No. 6473 of 23.04.13).

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