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PROJECT OF METHOD DEVELOPING OF QUALITY CONTROL OF SPRUCE ESSENTIAL OIL BY GAS-LIQUID CHROMATOGRAPHY

Conditions of the chromatography analyses of essential oils providing the yield of all components with the contents over 0.01% were selected. Essential oils of 10 samples of Norway spruce growing in the same edaphic-climatic conditions of the park UE "Brovki" were obtained by hydrodistillation. Qualitative and quantitative analyses of the received essential oils were performed. More than 50 components composing the essential oil were identified. The obtained statistic data allowed to calculate standard deviation of repeatability, standard deviation of intermediate precision with a variable factor "time + operator", expanded uncertainty of measurement results for the confidence level $P = 0,95$.

Introduction. Forest plays an important habitat-forming role. It influences on gas balance and composition of the atmosphere, water and thermal regime of the Earth's surface. It creates and stores topsoil, regulates the number and variety of wildlife. It not only gives a person a building material and gasoline, but also serves as a source of production of paper, turpentine, rosin, glycerol, detergents, resins, fodder yeast, conifer vitamin flour, tannins, essential oils, etc.

The total forest area of the Republic of Belarus is about 8 million hectares. Coniferous forests are most widely in Belarus. They occupy about 65% of the forested area. Common conifers are Scots pine (Lat. *Pinus sylvestris*) and spruce (Lat. *Picea abies L. H. Karst*).

Pine forests are distributed throughout the territory of the republic, but especially in the south of Belarus. Spruce forests are more common in the north of Belarus and occupy about 11% of the forested area [1].

A falsification of essential oils is recently a major problem both in Belarus and abroad due to the wide use of essential oils.

The falsification of natural essential oils means the premeditated changes of composition of essential oil for self-interested aims by mixing various additives and/or partial withdrawal of the most valuable components of essential oils while conserving some visible commercial product quality.

Synthetic additives, highly volatile (so-called turpentine fractions of some essential oils), cheaper essential oils, purified kerosene, vegetable fats and even mineral oils can be used as falsification products [2].

In the Republic of Belarus, the control over the essential oil products is carried out according to GOST (All-Union State Standard) 14618.10–78 "Essential oils, scented substances and intermediate products of their synthesis. Methods for determination of density and refractive index". Significant lack of quality control over the essential oils in Belarus is that the integrated features mostly carry it out. Refractive index that does not give reliable information about the composition of the essential oil.

Measurement procedure (MP) is the set of requirements for the methods, tools, processes for the preparation and processing of the measurement results of the observations that can provide the specified accuracies of measurement result under these conditions. It is known that the accuracy and reliability of the results depends not only on excellence measuring instruments but to a large extent on the correct procedures for their implementation.

Accuracy of the result can be evaluated by different characteristics:

– Correctness;
– Precision;
– Accuracy.

Precision is the scatter of results obtained under certain conditions. There are the following types of precision:

– Repeatability (closeness of the results in one laboratory under the same conditions);
– Intermediate precision (closeness of the results obtained in the same laboratory, but under different conditions).

Evaluation of the accuracy of results of the measurement precision (intermediate precision correctness) is carried out in accordance with STB ISO 5725 (Part 1–6) [3].

The need of considering the term "precision" arises from the fact that measurements performed on hypothetically identical materials under supposedly identical conditions, do not give, as a rule, identical results. It is explained not by random inevitable errors typical for each measuring procedure, and the factors that influence the measurement results cannot be completely controlled.

The precision depends only on the random errors and does not relate to the use of real or specified value of measurement quantity. Measure of precision is usually expressed in terms of inaccuracies and it is calculated as the standard deviation of the measurements. The quantitative measures of

precision values depend significantly on the regulated conditions. The extreme cases of such conditions are the conditions of repeatability and reproducibility conditions [3].

Thus, the main purpose of this research was the developing of techniques to determine the main components of the terpenoid composition of the essential oil of spruce.

Main part. Some essential oils obtained from the needles by hydrodistillation of 40 years spruce trees growing in the same soil and climatic conditions of UE "Brovki" arboretum were selected as an object of research.

Needle samples were selected from 10 trees in order to obtain statistical control of samples uniformity in the autumn and winter months of 2012, when the yield of essential oil reaches its maximum and its composition becomes stable [4].

The analysis of the composition of essential oil European spruce was performed by gas-liquid chromatography with *Crystal 5000.1* chromatograph using a quartz capillary column of 60 m long with pure dimethylsiloxane [5]. The experiment was carried out under the following chromatographic conditions: isothermal analysis at 70°C for 20 min with following programmed orthonormal temperature rise rate of 2°C/min to 150°C and the hold at final temperature for 40 minutes. Evaporator temperature is 250°C. Conductivity of the sample volume is 0.2 ml.

Each sample was chromatographed three times in order to obtain the statistic data on the quantitative content of the main components of the spruce essential oil.

It was found that the main compounds of spruce essential oil are tricycles, α-Pinene, Camphene, β-Pinene, Limonene, Camphor, Borneol, α-Terpineol, Borneolacetate.

Two main components of the monoterpene part of the spruce essential oil were chosen to develop the techniques: α-Pinene and Camphene. Identification of these components was performed using reference compounds [5, 6].

Essential oils derived from spruce wood greens were almost colorless, with characteristic balsamic odor of pine needles.

Individual composition of terpenes and their oxygenated in essential oils of spruce trees was not diverse and remained stable. The number of identified compounds in analyzed samples of essential oil components was 54 components with the total contribution of 92 wt %.

To develop a methodology the averages of α-Pinene and Camphene content were calculated. They range from 7.02 to 12.27 wt % and from 14.70 to 25.03 wt % respectively.

Accuracy specifications of the project methodology for α-Pinene and Camphene are presented in Table 1.

Measurement range, the relative values of the limits of repeatability and intermediate precision are given in Table 2.

The control of repeatability of parallel measurements is carried out by comparison of discrepancies between parallel measurements of mass concentration of α-Pinene and Camphene in the analyzed sample r_R,%, which is calculated by the formula (1), with a limit of repeatability r,% (results for two individual observations), given in Table 2.

$$r_R = \frac{(X_1 - X_2)}{X_{\text{av}}} \cdot 100\,\% \le r, \qquad (1)$$

where X_1 and X_2 are the results of the parallel measurements of the mass concentration of the component in the sample produced under repeatability when measuring by this method, wt % X_{avg} – the arithmetic mean of the results of two parallel measurements of the highest component concentration in the analyzed sample, wt %.

Table 1

Measurement range, relative values of repeatability, intermediate precision, the expanded uncertainty

Investigated compound	Measurement range, wt %	Mean root square deviation of frequency σ_r, abs. %	Mean root square deviation of the intermediate precision with variable factor «time + operator», $\sigma_{(TO)}$, abs. %	Expanded uncertainty of measurement result for confidence probability level P = 0,95, $\pm U$, abs. %
α-Pinene	6.8–12.4	0.003	0.005	0.020
Camphene	14.5–25.3	0.002	0.003	0.016

Table 2

Measurement range, relative values of repeatability and intermediate precision

Investigated compound	Measurement range (for two results of individual observations), r_R, %	Limit of intermediate precision (for two results of individual observations), $r_{(TO)}$, %
α-Pinene	0.008	0.014
Camphene	0.005	0.011

Several measurements of the same sample are performed in the process of the control of the intermediate precision. The measurements are carried out with maximum variation of conditions of measurement (two analysts are involved into the work, the measurements are made at different times). The intermediate precision of measurement work samples is taken during the controlled period. It is considered satisfactory if the following condition is kept:

$$\left|\overline{X_1} - \overline{X_2}\right| \le r_{(\mathrm{TO})}, \qquad (2)$$

where X_1 and X_2 are the results of measurements, wt %; $r_{(\mathrm{TO})}$ is the limit of intermediate precision with variable factor «time + operator»,% (according to Table 2).

The measures should be repeated if these conditions are changed. As a result of repeat violation the causes leading to unsatisfactory results of control must be found out and eliminated.

Conclusion. The spruce essential oil was produced on the base of 10 samples of spruce growing in natural conditions.

Qualitative and quantitative composition of produced essential oils was investigated. It was found that the qualitative composition of oil remains constant. The quantitative contribution of individual components varies slightly. It would be connected with the condition of the trees.

Mean root square deviation of frequency for α-pinene is σ_r = 0.003 abs. % and for camphene is σ_r = 0.002 abs. %. Mean Root Square Deviation of the Intermediate Precision with Variable Factor «time + operator» for α-Pinene is $\sigma_{(\mathrm{TO})}$ = 0.005 abs. % and for camphene is $\sigma_{(\mathrm{TO})}$ = 0.003 abs. %. Expanded uncertainty of measurement results for the confidence level P is 0.95, for α-Pinene is ± U = 0.020 abs. % and for camphene is ± U = 0.016 abs. %.

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