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Development of Methods of Analysis of Biological Active Substances for Standardization of Dense Extract of Creeping Thyme

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Abstract: Pharmaceutical development of mucolytic agent with dense extract of creeping thyme and essential oil of thyme involves obtaining a dense thyme extract of creeping, that is, choosing of optimal conditions that will allow in a short time to obtain a dense extract enriched with the main biological active substances (BAS) - which is a resultant extract and finished drug. Since in the analysis of creeping thyme herb in order to standardize this raw material as a quality index, among others, we chose the composition of flavonoids and hydroxycinnamic acids and the quantitative content of flavonoids, then it is appropriate to study the qualitative and quantitative composition of these bioactive substances and in the obtained extract. The aim of our work was to develop methods of analysis of BAS for the standardization of dense extract of creeping thyme; Identification and determination of the quantitative content of the analyzed biological active substances in herbal medicinal products and their respective extracts were carried out using modern sensitive and selective methods of analysis (thin-layer chromatography, differential spectrophotometry); As a result of TLC, the presence of fructose, glucose, arabinose, xylose, and rhamnose were found in the dense creeping thyme extract. According to the ratio of the size and intensity of staining on the chromatograms, it has been concluded that the predominant monosaccharide is fructose, and from the reducing monosaccharides - glucose, which will allow them to choose markers of the quality of the test extract. We also identified tyrosine, alanine, leucine, glycine, glutamic and aspartic acids at the levels of the respective zones on the chromatogram of the comparison solution. Chromatographic studies of a dense extract of creeping thyme by the thin-layer chromatography method have allowed identifying phenol carboxylic acids – rosmarinic (main representative), caffeic and chlorogenic; flavonoids – luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin, and rutin. Two acids were not identified, small quantities of which are found in the upper part of a chromatogram and of the unidentified structure of luteolin glucoside, which, by size and intensity of fluorescence, are not the main representative of flavonoids in the extract. Based on the literature, this representative of flavonoids may be Luteolin-7-O-di or triglycoside; Qualitative composition of a dense extract of creeping thyme herb in BAS groups – flavonoids, amino acids, polysaccharides has been studied. In order to standardize the extract, identification markers from phenolic compounds – luteolin-7-O-glucoside, luteolin, rutin, chlorogenic, rosmarinic, and caffeic acids, luteolin glycoside of unknown composition has been proposed. The quantitative content of flavonoids, amino acids, and reconstructive monosaccharides in the dense extract has been investigated. The correlation of content of the main BAS groups for dense extract indicates that the selected conditions for transferring the liquid extract to the dense are correct. When standardizing the dense extract of creeping thyme by the flavonoid content is proposed to be at least 2.5% in terms of apigenin.

Keywords: dense exract; creeping thyme; BAS groups; quantitative analysis; qualitative analysis.

1. Introduction

One of the priority directions of the modern pharmaceutical industry is the development of new drugs of plant origin [1-3]. The long-known herbal medicinal products (HMP), which remain relevant for the production of drugs, is a herb of creeping thyme (CT) [3, 4]. This development involves obtaining a dense extract, so the purpose of our work is to develop technology and standardize the dense extract of creeping thyme. Pharmaceutical development of mucolytic agent with dense extract of creeping thyme and essential oil of thyme involves obtaining a dense thyme extract of creeping, that is, choosing of optimal conditions that will allow in a short time to obtain a dense extract enriched with the main biological active substances (BAS) - which is a resultant extract and finished drug. Since in the analysis of creeping thyme herb in order to standardize this raw material as a quality index, among others, we chose the composition of flavonoids and hydroxycinnamic acids and the quantitative content of flavonoids, then it is appropriate to study the qualitative and quantitative composition of these bioactive substances (BAS) and in the obtained extract [7-10].

2. Materials and Methods

Identification and determination of the quantitative content of the analyzed BAS in HMP and their respective extracts were carried out using modern sensitive and selective methods of analysis. The qualitative composition of flavonoids was determined by the thin-layer chromatography method [11, 12] in the solvent system *ethyl acetate P – formic acid P – water P* (90:6:9). The determined volumes of solutions were applied to chromatographic plates Silica gel F_{254} of company "Merck" using a device for automatic sampling on "CAMAG Linomat 5" plate, chromatography was carried out in a "GAMAG" chromatography chamber. Approaching the standardization of liquid and dense thyme creeping extract, we considered the possibility of identifying the same BAS, continuing the proposed approach in the HMP chain – extracts [9]. The determination of the amount of flavonoids was carried out by the method of differential spectrophotometry on the spectrophotometer of the brand "Cary-50" [12]. The quantitative content of flavonoids in the dense extract was determined by the reaction of the formation of the colored compound directly of flavonoids with aluminum chloride. To solve the problems a liquid extract of CT has been used, the technology of which has been described by us in the patent of Ukraine on the utility model No. 73543 [5] and the following equipment: a deep vacuum VT6 pump with a maximum dilution of up to 0.85 kp/cm^2 with vacuum gauge ОБВ 1-300 with range measurement from 0 to -1 kp/cm^2; laboratory rotary evaporator LABOROTA 4001.

During the experiment, we took 500 ml of a creeping thyme extract. Evaporation was carried out to a volume of 100 ml fixing the time of evaporation under the following conditions:
- temperature value 60 ^{0}C, vacuum 0,6 kp/cm^2;
- temperature value 60 ^{0}C, vacuum 0,8 kp/cm^2;
- temperature value 80 ^{0}C, vacuum 0,6 kp/cm^2;
- temperature value 80 ^{0}C, vacuum 0,8 kp/cm^2.

Modes of the inspissation of liquid extract of creeping thyme results are presented in Table 1.

Table 1. Modes of inspissation of liquid extract of creeping thyme.

Mode	**Temperature, ^{0}C**			
	60		**80**	
Vacuum, kp/cm^2	0,6	0,8	0,6	0,8
Time of evaporation, min. *(from 500 ml to 100 ml)*	95	80	65	55

From the data obtained, it can be concluded that the temperature of evaporation is essentially influenced, that it is about 50% faster, evaporate the extract at 80°C, compared to 60°C of evaporation. With a vacuum dilution of 0.8 kp/cm^2, the gain is about 15-20% in comparison with the vacuum of 0.6 kp/cm^2.

The technological process of producing dense creeping thyme extract consists of the following stages:

Stage 1. Sanitary preparation of production.

Stage 2. Preparation of raw materials

Stage 3. Obtaining a liquid extract from a creeping thyme herb.

Stage 4. Obtaining extract of dense creeping thyme.

Sanitary preparation of production includes the following operations:

Stage 1.1. preparation of disinfectant solutions;

Stage 1.2. preparation of ventilation air;

Stage 1.3. preparation of production premises;

Stage 1.4. preparation of equipment and inventory;

Stage 1.5. preparation of workwear;

Stage 1.6. training of work personnel.

Extract of dense thyme creeping from a vacuum-evaporation devise pos. P81 is unloaded into a clean, dry polyethylene barrel. The controller of QCD of the enterprise selects a sample of finished products for analysis on compliance with the requirements of Quality Control Techniques No.UA/6809/01/01 (K4.2.7).

Identification and determination of the quantitative content of the analyzed BAS in HMP and their respective extracts were carried out using modern sensitive and selective methods of analysis. The qualitative composition of flavonoids was determined by the thin-layer chromatography method [11, 12] in the solvent system *ethyl acetate P – formic acid P – water P* (90:6:9). The determined volumes of solutions were applied to chromatographic plates Silica gel F_{254} of company "Merck" using a device for automatic sampling on "CAMAG Linomat 5" plate, chromatography was carried out in a "GAMAG" chromatography chamber. Approaching the standardization of liquid and dense thyme creeping extract, we considered the possibility of identifying the same BAS, continuing the proposed approach in the HMP chain – extracts [9]. As a result of conducted thin-layer chromatography studies, we propose the following method for the identification of dense extract of creeping thyme.

Method for dense identification extract of creeping thyme:

Test solution: 0.4 g of a dense extract of creeping thyme is dissolved in 10 ml of 96% *ethanol* P in an ultrasonic bath for 10 minutes, filtered.

Blank solution. 0.5 mg of RS of rosmarinic acid (Fluka), 0.5 mg of RS of caffeic acid (Fluka), 0.5 mg of RS of chlorogenic acid (Fluka), 1.0 mg of PhRS of luteolin-7-O-glucoside (SPhU), 0.5 mg of RS of rutin (Sigma) and 0.5 mg of luteolin (Fluka) are dissolved in 20 ml of methanol R.

On the line of the start of the chromatographic plate, 10 µl of the *test solution* and 5 µl of a *blank solution* are applied separately by strips of 10 mm. The plate is placed in the chamber with a mixture of solvents *formic acid anhydrous P-water P-ethyl acetate P* (6:9:90). When the front of the solvents passes 10 cm from the start line, the plate is removed from the camera.

Drying: dried in the air, and then kept at a temperature from 100ºC to 105ºC for 2 minutes.

Detection: A warm plate is sprayed with a *solution of 10 g/l of the aminomethyl ether of diphenylboronic acid P in methanol P,* dried in the air. The plate is then sprayed with a solution of *50 g/l macrogol 400 P in methanol P*, dried in the air for 30 minutes, and revised in UV light with a wavelength of 365 nm.

Results: on chromatogram of *blank solution* should be determined (in the order of R_f growth): yellow-orange fluorescence zone, corresponding to rutin (R_f = 0.18), blue fluorescent zone, corresponding to chlorogenic acid (R_f = 0.32); yellow-orange fluorescent zone corresponding to luteolin-7-O-glucoside (R_f = 0.42), light green-blue fluorescent zone, corresponding to rosmarinic acid (R_f = 0.80), blue fluorescent zone, corresponding to caffeic acid (R_f = 0,82), yellow-orange fluorescent zone, corresponding to luteolin (R_f = 0.84).

On the chromatogram of the *test solution* should be detected six zones (rutin, chlorogenic acid, luteolin-7-O-glucoside, rosmarinic acid, caffeic acid, and luteolin) at the level of zones on the chromatogram of blank solution corresponding to their coloring and fluorescence and the bright yellow-orange zone of fluorescence (R_f = 0.29) below the zone of chlorogenic acid on the chromatogram of blank solution. Other zones of blue and yellow-orange fluorescence may be detected.

The sequence of zones on the chromatograms of *test solution* (dense extract) and a blank solution is shown in Picture 2 below.

Method for the identification of monosaccharides in a dense extract of creeping thyme.

Test solution: 0.4 g of dense extract was placed in a centrifuge tube, 10 ml of *water P* was added and left in an ultrasonic bath for 10 min, and then centrifuged. The supernatant fluid was transferred to a 100-ml glass, 30 ml of 96% alcohol was added. The solution was then settled for 1 hour, centrifuged, decanted, and the following operations were performed with precipitate: was added 5 ml of acid 2 mol/l sulfate and transferred quantitatively to a 50 ml flask with slide. Heating was conducted in a water bath under reflux for 1 hour. After the hydrolysis, the resulting solution was neutralized by carbonate barium. Later the mixture was centrifuged, the precipitate was removed, and the supernatant fluid was used as the test solution.

Blank solution: 10 mg of standard samples of fructose, glucose, arabinose, galactose, xylose, and rhamnose were placed in a volumetric flask and dissolved in *water P,* bringing the solution volume with *water P* to the mark.

To the starting line of the chromatographic plate, “Silica gel” with size 20 x 20 cm (Merck, Germany) is applied 15 µl of the test solution and 5 µl of blank solution. The plate is dried in air for 10 minutes, placed in a mobile phase chamber *water P – acetonitrile P* (15:85), and chromatographed by an ascending method. When the front of solvents passes 15 cm from the start line, it is taken out of the chamber and dried in the air. The plate is sprayed with a solution of thymol (0.5 g of thymol, 5 ml of concentrated sulfuric acid, and 95 ml of *96% of alcohol P*) and heated at a temperature of 100-105°C for 3-5 minutes, revised in daylight.

As a result, the presence of fructose, glucose, arabinose, xylose, and rhamnose in a dense extract of creeping thyme was established. By the ratio of the size and intensity of staining on chromatograms, it was concluded that the predominant monosaccharide is fructose, and from reconstructive monosaccharides – glucose. Thus, it is suggested that the presence of fructose and glucose should be chosen as markers of the quality of the test extract.

In order to prove the presence of amino acids in the corresponding extract, thin-layer chromatography analysis was performed [14].

The method for identifying amino acids in a dense extract of creeping thyme is given below.

Test solution: 0.4 g of the dense extract is dissolved in 10 ml *of alcohol (30%, v/v) P* in an ultrasonic bath for 10 minutes.

Blank solution: 10 mg of standard samples of glycine, leucine, tyrosine, alanine, aspartic acid, and glutamic acid are dissolved in 25 ml of *water P*.

On the starting line of chromatographic plate Silica gel F_{254} with size, 20x20 cm with a thickness of 0.25 mm is applied with strips of 10 mm in length 20 µl of the test solution and 5 µl of blank solution. The plate is dried in the air for 30 min, placed in a mobile phase chamber with *isopropanol P - formic acid P - water P* (40: 2: 10) and chromatographed by ascending method. When the front of solvents passes 15 cm from the start line, it is taken out of the chamber and dried in the air. The plate is sprayed with a *solution of ninhydrin P* in a mixture of *acetic acid P* and *butanol P*, heated at a temperature of 100-105ºC for 3-5 minutes and viewed in daylight.

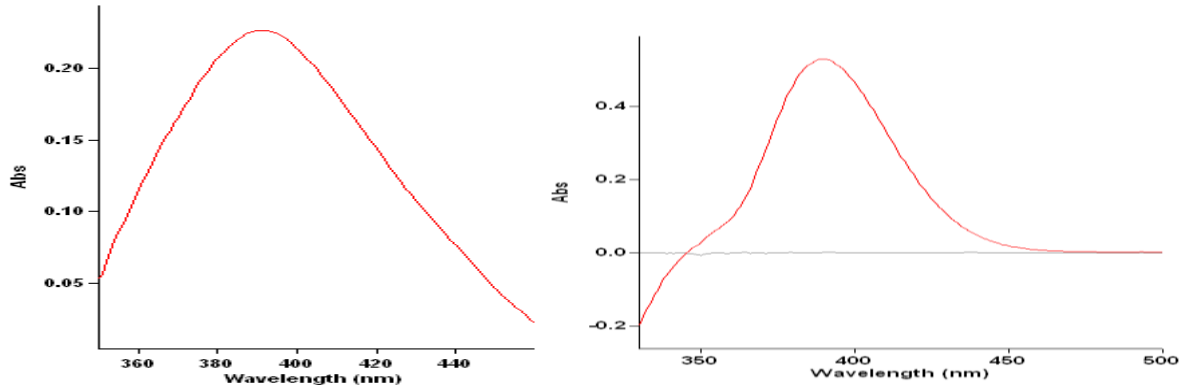
On the chromatograms of the tested solution of dense extract, zones of tyrosine, alanine, leucine, glycine, glutamine, and aspartic acids were observed at the level of corresponding zones on the chromatogram of comparison solution.

The next stage of the study was the identification of polysaccharides in a dense extract of creeping thyme and performance using the thin-layer chromatography method on the plates "Silica gel" (Merck, Germany) in the solvent system *water P-acetonitrile P* (15:85) using a solution of RS of monosaccharides (arabinose, galactose, rhamnose, fructose, glucose, xylose). Chromatograms were indicated by the solution of thymol (0.5 g of thymol, 5 ml of concentrated sulfuric acid, and 95 ml of 96% ethyl alcohol).

The determination of the amount of flavonoids was carried out by the method of differential spectrophotometry on the spectrophotometer of the brand "Cary-50" [12].

Since apigenin was selected as the standard for calculating their content in raw materials, then when controlling the quality of extracts, it is necessary to calculate the number of flavonoids on apigenin [9].

Differential electronic absorption spectra of a complex of aluminum chloride with flavonoids, present in the dense extract of creeping thyme, are characterized by the presence of a maximum of absorption at 390 ± 2 nm (Fig. 1).



a) b)

Figure. 1. Differential electronic absorption spectra of test (a) and blank solution (b) under the quantitative determination of flavonoids in a dense extract of creeping thyme (λ_{max} = 390,0 nm).

As can be seen, in terms of quantitative determination of flavonoids, the absorption spectrum of test solution for the studied dense extract of creeping thyme along the course of the curve and the position of maximum corresponds to the absorption spectrum of the

corresponding complex of apigenin. Therefore the amount of flavonoids in the extract was calculated in terms of apigenin.

We propose the following procedure to determine the amount of flavonoids in a dense extract of creeping thyme.

Method for determining the amount of flavonoids in a dense extract of creeping thyme.

Initial solution: 0.13 g (exact sample weight) of a dense extract of creeping thyme is placed in a 25 ml volumetric flask, dissolved in 20 ml of *alcohol (60%, v/v) P*, and the volume of solution is adjusted to the mark with the same alcohol, then mixed.

Test solution: 1.0 ml of *initial solution* is placed in a 25 ml volumetric flask, 2.0 ml of 3% solution of aluminum chloride is added and adjusted with *alcohol (70%, v/v) P* to the mark, then mixed.

Compensation solution: 1.0 ml of *initial solution* is placed in a 25 ml volumetric flask, and the volume of solution is adjusted with *alcohol (70%, v/v) P* to the mark, then mixed.

Standard solution of apigenin: 0,03 g (exact sample weight) of a standard sample of apigenin (Fluka) is placed in a 100 ml volumetric flask, 70 ml of *alcohol (70%, v/v) of P* is added, dissolved, and the volume of solution is adjusted to the mark with the same solvent and then mixed.

10.0 ml of the resulting solution is placed in a 25 ml volumetric flask, and the volume of solution is adjusted with *alcohol (70%, v/v) P* to the mark, then mixed.

Blank solution: 1.0 ml of a solution of a standard sample of apigenin is placed in a 25 ml volumetric flask, 2.0 ml of 3% aluminum chloride solution is added, and the volume of solution is adjusted with *alcohol (70%, v/v) P* to the mark, then mixed.

Compensation solution: 1.0 ml of a solution of a standard sample of apigenin is placed in a 25 ml volumetric flask, and the volume of solution is adjusted with *alcohol (70%, v/v) P* to the mark, then mixed.

After 45 minutes, differential electronic absorption spectra are recorded for the *test solution* and *blank solution* relative to compensation solutions for each, respectively, and the optical density is measured at the absorption maximum at a wavelength of 390 ± 2 nm.

Method of quantitative determination of the content of reconstructive monosaccharides in a dense extract of creeping thyme.

Initial solution: 0.2 g (exact sample weight) of a dense extract of creeping thyme is placed in a 25 ml volumetric flask, added 10 ml of *water P*, dissolved and placed in a centrifuge tube of 50 ml capacity, added 30 ml of *96% alcohol P*, mixed and heated in a water bath at a temperature of 30ºC for 5 minutes. Maintained for 1 hour, the content is centrifuged, the supernatant fluid is discarded, and the precipitate is transferred with 5.0 ml of acid of diluted hydrochloric P and 5.0 ml of water P in a conical flask of 50 ml capacity with slide and heated for 1 hour with a reflux condenser in boiling water bath. Flask with content is cooled, placed a small piece of congo paper in a flask and added 40% sodium hydroxide solution to reddening the paper drop by drop, then added drops of hydrochloric acid diluted to the paper, and then drop by drop 10% sodium hydroxide solution until the paper reddened. The resulting solution is transferred quantitatively with water P into a volumetric flask of 25 ml capacity, brought the volume of solution to the mark with water P, mixed and filtered, discarding the first 5 ml of filtrate.

Test solution: 1.0 ml of 1% citric acid solution is placed in a 25 ml flat bottom flask, added 3.0 ml of 20% solution of sodium carbonate, and 1.0 ml of solution B. The flask with content is kept in a boiling water bath for 10 minutes, then cooled to room temperature. The

content of the flask is transferred quantitatively with *water P* into a volumetric flask of 25 ml capacity and brought the volume of solution to the mark with *water P*.

Standard sample of glucose: 0.14 g (exact sample weight) of a standard sample of glucose (Fluka), dried at a temperature of 100 to 105ºC to a constant mass, is placed in a volumetric flask of 100 ml capacity, dissolved in 50 ml of *water P*, adjusted the volume of solution with *water P* to the mark and mixed. 10,0 ml of the resulting solution is placed in a 25 ml volumetric flask, adjusted the volume of solution with *water P* to the mark, and mixed.

Blank solution: 1.0 ml of 1% picric acid solution, 3.0 ml of 20% solution of sodium carbonate, and 1.0 ml of a *solution of standard glucose sample*, treated similarly to the test solution, starting with the words: "The flask with contents is adjusted...".

Compensation solution: 1.0 ml of 1% picric acid solution, 3.0 ml of 20% sodium carbonate solution, and 1,0 ml of *water P*, treated similarly to the test solution, starting with the words: "The flask with contents is adjusted ...".

The optical density of the *test solution* and a *blank solution* is measured at a wavelength of 460 nm relative to the compensation solution.

Method of quantitative determination of the content of amino acids in a dense extract of creeping thyme.

Test solution: 0.13 g (exact sample weight) of a dense extract of creeping thyme is placed in a 25 ml volumetric flask, dissolved in 20 ml of *alcohol (30%, v/v)*, and brought the solution volume to the mark with the same alcohol, then mixed.

1.0 ml of the resulting solution is treated in a similar manner to the test solution placed in the test tube, 1,1 ml of freshly prepared 0,2% *ninhydrin solution P* is added, and heated in a boiling water bath for 20 minutes. After complete cooling, transferred the solution quantitatively into a volumetric flask of 100 ml capacity and diluted with water P to the mark, mixed.

Blank solution: 0.059 g (exact sample weight) PhRS glycine is placed in a volumetric flask of 100 ml capacity, 70 ml of *water P* is added, dissolved for 5-10 minutes, heated in a water bath at a temperature of 50 °C. Cooled and brought the volume of solution with *water P* to 100 ml and mixed.

To 1.0 ml of received solution, placed in the test tube, is added 1.1 ml of 0.2% solution of *ninhydrin P* and heated in a boiling water bath for 20 minutes. After complete cooling, the solution is transferred quantitatively into a volumetric flask of 100 ml capacity and diluted with *water P* to the mark, mixed.

Compensation solution: 1.0 ml of *water P* is placed in the test tube, is added 1.1 ml of 0.2% solution of *ninhydrin P*, and heated in a boiling water bath for 20 minutes. After complete cooling, the solution is transferred quantitatively into a volumetric flask of 100 ml capacity and diluted with *water P* to the mark, mixed.

0,2% solution of ninhydrin P: 0.1 g of ninhydrin is placed in a 50 ml volumetric flask, dissolved in 30 ml of *water P*, the volume of solution is adjusted to the mark with the same solvent and mixed.

After 1 hour, the optical density of the test solution and blank solution on a spectrophotometer at a wavelength of 567 ± 2 nm in a cuvette with a thickness of 10 mm relative to the compensation solution is measured.

3. Results and Discussion

The result, the finished product was received – a dense extract of creeping thyme herb, which is a dense bundle of mass that does not pour out of the container but extends into the filaments and once again fuses into a solid mass with a specific odor. Chromatographic studies of a dense extract of creeping thyme by the thin-layer chromatography method have allowed identifying phenol carboxylic acids – rosmarinic (main representative), caffeic and chlorogenic; flavonoids – luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin, and rutin. Two acids were not identified, small quantities of which are found in the upper part of a chromatogram and of the unidentified structure of luteolin glucoside, which, by size and intensity of fluorescence, are not the main representative of flavonoids in the extract. Based on the literature [13], this representative of flavonoids may be luteolin-7-O-di or triglycoside.

As a result of conducted thin-layer chromatography studies (Fig. 2), the presence of flavonoids and phenol carboxylic acids in a dense extract of creeping thyme has been proved, which would allow to objectively establish the identity of investigated extracts for the presence of BAS of used raw material.

Upper part of the plate	
Luteolin: yellow-orange fluorescent area	yellow-orange fluorescent area (luteolin)
Caffeic acid: blue fluorescence zone	blue fluorescence zone (caffeic acid)
Rosmarinic acid: light green-blue fluorescence zone	light green-blue fluorescence zone (rosmarinic acid)
Luteolin-7-O-glucoside: yellow-orange fluorescence zone	yellow-orange fluorescence zone (luteolin-7-O-glucoside)
Chlorogenic acid: blue fluorescent zone	blue fluorescent zone (chlorogenic acid) *very intense* yellow-orange fluorescence zone
Rutin: yellow-orange fluorescent area	yellow-orange fluorescent area (rutin)
Test solution	Blank solution

Figure 2. Scheme of the chromatogram in conditions of identification of dense extract of creeping thyme after treatment with solutions of an amine-ethyl ester of diphenylboronic acid and macrogol 400 when viewed in UV light with a wavelength of 365 nm.

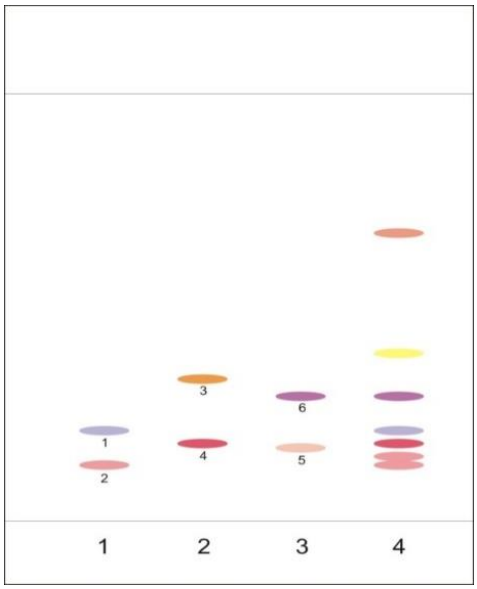


Figure 3. Scheme of the chromatogram of a dense extract of creeping thyme (4) and blank solutions (1, 2, 3, where 1 – arabinose, 2 – galactose, 3 – rhamnose, 4 – fructose, 5 – glucose, 6 – xylose) in the conditions of identification of monosaccharides

As a result of the chromatographic study (Fig. 3), we identified seven monosaccharides, one of which remained unknown due to the lack of a required standard.

The scheme of the chromatogram obtained during the study of a dense extract of creeping thyme in terms of the identification of amino acids is shown in Figure 4.

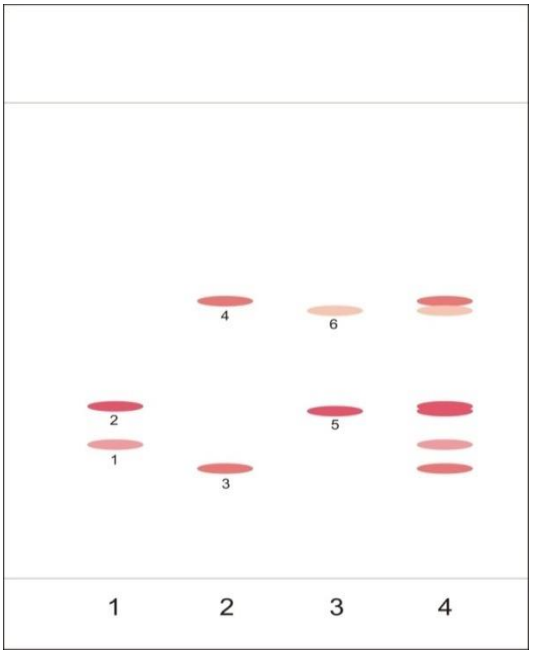


Figure 4. Scheme of the chromatogram of the test solution (4) and blank solutions (1, 2, 3), in which 1 – glycine; 2 – alanine; 3 – aspartic acid; 4 – leucine; 5 – glutamic acid; 6 – tyrosine.

Results of the quantitative determination of the amount of flavonoids in the dense extract are given in Table 2.

Table 2. Results of determination the content of flavonoids in dense extract (P = 0.95, n = 5).

	Content of flavonoids in terms of apigenin, %			
	No.1	**No.2**	**No.3**	**No.4**
Dense extract of creeping thyme	2.81 ± 0.01	2.80 ± 0.02	2.81 ± 0.03	2.77 ± 0.02

Given the results of quantitative determination, standardizing the dense extract can be proposed as a quality criterion for the content of flavonoids-not less than 2,5% in terms of apigenin [9-12].

The quantitative content of reconstructive monosaccharides (Table 3) in the investigated extract varies and is determined by the content in the raw material and the reproducibility of the selected extract technology.

Table 3. Results of determination of the content of reconstructive monosaccharides in a dense extract of creeping thyme (P = 0,95, n = 5).

Extract	**Content of reconstructive monosaccharides in terms of glucose, %**			
	No.1	**No.2**	**No.3**	**No.4**
Dense extract of creeping thyme	6.02 ± 0.10	6.87 ± 0.09	5.60 ± 0.10	6.97 ± 0.11

Determination of amino acids in the obtained extract of creeping thyme was also performed by spectrophotometry in the visible spectral region using the well-known and used photometric reaction of the formation of a colored compound of amino acids with ninhydrin (Table 4) [14-16].

Table 4. Results of quantitative determination of the amount of amino acids in dense extract of creeping thyme (P = 0,95, n = 5).

Extract	Content of amino acids in terms of glycine, %			
	No.1	No.2	No.3	No.4
Dense extract of creeping thyme	1.86 ± 0.02	2,18 ± 0.01	1,50 ± 0.01	2, 08 ± 0.02

Therefore, acids – rosmarinic, caffeic, chlorogenic and flavonoids - luteolin-7-O-glucoside, rutin, and luteolin, as well as the unidentified composition of luteolin glucoside, as the identity markers of a dense extract of creeping thyme, the presence of which zones on the chromatogram of the investigated object will allow it to be objectively identified [11].

The quantity of flavonoids in the investigated extract varies within certain limits and is determined by their content in the raw material and the reproducibility of extract technology. Given the results of quantitative determination, standardizing the dense extract can be proposed as a quality criterion for the content of flavonoids-not less than 2,5% in terms of apigenin [9-12]. The quantity of flavonoids in the investigated extract varies within certain limits and is determined by their content in the raw material and the reproducibility of extract technology.

Guided by the results of the quantitative determination of reconstructive monosaccharides, when standardizing the dense extract of creeping thyme, the quality of the content of reconstructive monosaccharides can be proposed to be at least 5.0% in terms of glucose.

Thus, the quantitative content of the amount of amino acids in terms of glycine in the studied extract of creeping thyme has been determined as a quantitative quality criterion has been proposed their content of at least 1,0% in the dense extract, respectively.

4. Conclusions

The qualitative composition of a dense extract of creeping thyme herb in BAS groups – flavonoids, amino acids, polysaccharides has been studied. In order to standardize the extract, identification markers from phenolic compounds – luteolin-7-O-glucoside, luteolin, rutin, chlorogenic, rosmarinic, and caffeic acids, luteolin glycoside of unknown composition has been proposed. The quantitative content of flavonoids, amino acids, and reconstructive monosaccharides in the dense extract has been investigated. The correlation of content of the main BAS groups for dense extract indicates that the selected conditions for transferring the liquid extract to the dense are correct. When standardizing the dense extract of creeping thyme by the flavonoid content is proposed to be at least 2.5% in terms of apigenin.

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Conflicts of Interest

The authors declare no conflict of interest.

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