Resume: S.D. Asfendiyarov KazNMU has developed an original technology for obtaining Zeravshan thyme extract. A method for obtaining the extract using ultrasound with a regulated frequency is substantiated, which makes it possible to get the maximum yield of biologically active substances from medicinal plant materials with established pharmaceutical and technological parameters. The technology of three pilot batches was transferred at the pharmaceutical company FitOleum LLP to GMP. Technological process validation was successful. Technological regulations for production, quality specification "Seravshan thyme liquid extract" and a draft regulatory document have been developed. Studies have begun on long-term stability testing of the product. The developed method for obtaining the extract is patented (patent No. 6830 dated February 4, 2022).

Keywords: Seravschan thyme (Thymus seravschanicus), extract, technological process, raw material standardization, technology transfer, stability.

DEVELOPMENT OF TECHNOLOGY FOR OBTAINING EXTRACT OF ZERAVSHAN THYME (THYMUS SERAVSCHANICUS KLOKOV) AND ITS STANDARDIZATION

B.S. Zhumakanova¹, Z.B. Sakipova¹, L.N. Ibragimova¹, A.A. Kessikova², M.B. Ibrayeva¹, S.V. Shvets¹, A.A. Bakytkhanova¹
¹S.D. Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan
²Abbott Kazakhstan LLP, Almaty, Kazakhstan

ZЕРАВШАН ЖЕБІР СЫҒЫНДЫСЫН (THYMUS SERAVSCHANICUS KLOKOV) АЛУ ТЕХНОЛОГИЯСЫН ӘЗІРЛЕУ ЖӘНЕ ОНЫ СТАНДАРТТАУ


Разработана оригинальная технология получения экстракта тимьяна зеравшанского. Обоснован способ получения извлеченя с применением ультразвука с регламентируемой частотой, позволяющий получить максимальный выход биологически активных веществ из лекарственного растительного сырья с установленными фармацевтико-технологическими параметрами. Произведен перенос технологии трех пилотных серий на фармацевтическом предприятии ООО «ФитОлеум» в GMP. Валидация технологического процесса проведена успешно. Разработан технологический регламент на производство, спецификация качества «Экстракт тимьяна зеравшанского» и проект нормативного документа. Нача- ты исследования долгосрочных испытаний стабильности продукта. Разработанный способ получения экстракта запатентован (патент № 6830 от 04.02.2022 г.).

Ключевые слова: тимьян зеравшанский (Thymus seravschanicus), экстракт, технологический процесс, стандартизация сырья, перенос технологии, стабильность.
Introduction. In medical practice, the following pharmacopoeial plant species are currently official: common thyme (Thymus vulgaris L.) [1] and creeping thyme (Thymus serpyllum L.) [2–3]. Herbal pharmaceutical substances derived from them are part of dosage forms used as an expectorant, cough softener, antibacterial, antifungal and antioxidant, sedative, anthelmintic [4–6]. Thymus seravschanicus Klokov and Thymus marshellianus Wild are of scientific and practical interest to the scientists of S.D. Asfendiyarov Kazakh National Medical University. These plant species grow on the territory of the Dzungarian Alatau (the Republic of Kazakhstan), and there are sufficient reserves of them. In this regard, the School of Pharmacy conducts full-scale scientific research on Thymus seravschanicus and Marshall thyme. It has been established that these plant species have a pronounced antibacterial activity against some gram-negative bacteria, including Helicobacter pylori, and gram-positive bacteria, including streptococci and fungi. Test extracts were evaluated for reference microorganisms from the American Type Culture Collection (ATCC), including Gram-negative bacteria (Escherichia coli ATCC25922, Salmonella typhimurium ATCC 14028), Helicobacter pylori ATCC 43504, fungal strains (Candida albicans ATCC 10231, Candida parapsilosis ATCC 22019, gram-positive bacteria (Bacillus cereus ATCC 10876, Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923) [7, 8].

The phytochemical composition of Thymus seravschanicus has been established: essential oils (carvacrol, thymol, linalool, cineol, etc.); flavonoids (luteolin 7-O-glucuronide, luteolin 7-O-rutinoside, apigenin 7-O-glucuronide, eriodictyol, naringenin); triterpenes (ursolic and oleanolic acids); phenolic acids (gallic acid, protocatechin acid, caffeic acid, ferulic acid, rosmarinic acid, quinic and 5-methoxysaliclic acid hydrates); tannins, bitterness and others. The content of micro and macro elements, such as iron, molybdenum, selenium and boron, was also determined. It should be noted that the qualitative and quantitative composition of biologically active substances in Thymus seravschanicus is much higher compared to other species of the genus Thymus L. [7, 8].

The purpose of this work is to develop an optimal technology for obtaining Thymus seravschanicus extract and its standardization in accordance with pharmacopoeial requirements.

Materials and methods. For the production of a liquid extract, pharmacopoeial-quality medicinal plant raw materials (MPM) from herb Thymus seravschanicus, collected on the territory of the Dzungarian Alatau in accordance with the requirements of GACP [9], purified water and ethanol 96% are used. The technological process consists of the following stages and operations: preparation of medicinal plant materials, preparation of the extractant, obtaining an extract from the MPM using ultrasound, purification of the extract, removal of the extractant, packaging and labeling (Figure 1). The dried raw material of Thymus seravschanicus is crushed on a grass cutter to a particle size of not more than 5 mm, loaded into an ultrasonic extractor with a false bottom and a thermal jacket, poured with an ethanol solution with a concentration of 35-45% in the ratio of raw material-extractant 1: (7–10) and moistened for 3 to 5 hours. Then, the extractant is poured (ethyl alcohol solution 60–70%) to the mirror and extraction is carried out under ultrasonic action with a regulated frequency of 20–25 kHz for 15-20 minutes at a temperature of 30–35 °C. The resulting extract is poured into the storage tank. The extraction process is repeated at least three times, combining the obtained extracts. The combined extracts are centrifuged, the supernatant is decanted and the extractant is removed under vacuum at a temperature of 50 °C to 60 °C to a liquid extract (1:2). The resulting liquid extract is poured into class I brown glass bottles with a screw neck. Finished products in the form of a liquid extract of Thymus seravschanicus meet pharmacopoeial requirements.

In the production process, qualified technological equipment was used: a grass cutter, a sieve, scales, an ultrasonic extractor with a false bottom and a thermal jacket, a packing line, a centrifuge, and a vacuum evaporator. Standardization of the liquid extract of Thymus seravschanicus was carried out in accordance with the pharmacopoeial requirements for the following indicators: description, identification, relative density, ethanol content, dry residue, heavy metals, and the volume of the contents of the package, microbiological purity, and quantitative determination. The applied analytical methods have passed the verification/validation stage [1, 10]. The identification of thymol and carvacrol is conducted by TLC in accordance with the requirements of [10, 2.2.27]. On the chromatogram of the test solution, one adsorption zone with an Rf value of about 0.97 should be detected, which fluoresces brown-pink at the level of the thymol adsorption zone on the chromatogram of the reference solution, corresponding to it in size and shape, and a pale violet staining zone with an Rf value of about 0.89 at the level of the carvacrol adsorption zone in the chromatogram of the reference solution. The quantitative determination of the amount of phenolic compounds is carried out by absorption spectrophotometry in the UV and visible areas in accordance with the requirements of [10, 2.2.25].

Statistical processing of the results was carried out in accordance with the requirements of the SPPh RK [10]. The Minitab version 21 electronic program was used for the calculation. All data are presented as mean ± RSD.

Results and discussion. A technology has been developed for obtaining a liquid extract according to a technology with established pharmaceutical and technological parameters and ultrasound values with a regulated frequency of 20-25 kHz [7].

Criteria for the quality of the finished product and quality specification have been developed. The standardization of the finished product in accordance with pharmacopoeial...
**Figure 1** - Flow chart for the production of a liquid extract of *Thymus seravschanicus* from raw materials

**Table 1** - Quality specification for the extract of the herb *Thymus seravschanicus* Klokov

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Deviation rates</th>
<th>Test methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Liquid extract from brown to dark brown or brown in color, with a specific odor</td>
<td>SPh RK I, v. 1</td>
</tr>
<tr>
<td>Identification</td>
<td>The sequence of zones on the chromatograms of the reference solution and the test solution. Additional zones are allowed in the lower third of the chromatogram of the test solution - a brown-pink coloring zone is observed; - pale-violet zone staining.</td>
<td>TLC, V.1.2.2.27</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Not less than 60 %</td>
<td>SPh RK I, v. 1, 2.8.16</td>
</tr>
<tr>
<td>Dry residue</td>
<td>Not less than 8.0 %</td>
<td>SPh RK I, v. 1, 2.9.10</td>
</tr>
</tbody>
</table>
requirements was carried out and long-term stability tests were started. Table 1 presents the quality specification for the extract of the herb Thymus seravschanicus Klokov. Critical parameters were determined, risks were assessed by ICH Q 9 guidelines, a validation plan was developed and the process was validated on the following series: EZhTS-2018.1, EZhTS-2018.2, and EZhTS-2018.3. It has been established that all the studied critical parameters, including the worst case, are within the regulated limits (including 6 δ); the relative standard deviation does not exceed 2 %, the process is stable over time and complies with the ratio (Ср ≥ Срk ≥ 1.3); the process under study is valid.

**Microbiological purity**
The drug must comply with the requirements of the SPh RK I, v. 1, 5.1.4, category 3 B.

**SPh RK I, v. 1, 2.6.12 and v. 2, 2.6.13**

- The total number of aerobic bacteria is not more than 10,000 per 1 g.
- The total number of mushrooms is not more than 100 per 1 g or ml.
- Not more than 100 enterobacteria and some other gram-negative bacteria in 1 g.
- Absence of Salmonella in 10 g.
- Absence of Escherichia coli, Staphylococcus aureus in 1 g.

**Quantitation**
- amount of phenolic compound in terms of gallic acid
- Not less than 2 %

**SPh RK I, v. 1, 2.2.25**

**Package**
- 10 g of the drug in glass vials for medicines made of brown glass class I (SPh RK I, vol. 1, 3.2.1) with a screw neck

**SPh RK I, v. 1, 2.6.12 and v. 2, 2.6.13**

**Marking**
- See approved packaging layout

**SPh RK I, v. 1, 2.6.12 and v. 2, 2.6.13**

**Transportation**
- In accordance with GOST 17768-90

**Storage**
- In a place protected from light, at a temperature not exceeding 25 °C

**SPh RK I, v. 1, 2.6.12 and v. 2, 2.6.13**

**Shelf life**
- 2 years

**SPh RK I, v. 1, 2.6.12 and v. 2, 2.6.13**

**Main pharmacological action**
- Anti-inflammatory, antibacterial agent

**REFERENCES**

Authors' Contributions. All authors participated equally in the writing of this article. No conflicts of interest have been declared.

This material has not been previously submitted for publication in other publications and is not under consideration by other publishers. There was no third-party funding or medical representation in the conduct of this work. Funding - no funding was provided.

Author information:

Б. С. Жұмақанова, магистр, ассистент кафедры инженерных дисциплин и надлежащих практик, Казахский национальный медицинский университет имени С.Д.Асфендиярова, ул. Толе би 94, г. Алматы, Республика Казахстан; телефон: +7 705 578 21 27, e – mail: bagdush@mail.ru

З. Б. Сакипова, профессор, доктор фармацевтических наук, декан Школы фармации, Казахский Национальный медицинский университет имени С.Д.Асфендиярова, ул. Толе би, 94; Алматы, Республика Казахстан; телефон: +7 777 235 02 02, e – mail: sakipova.z@kaznmu.kz.

Л.Н. Ибрагимова, доцент, курс технологии лекарственных средств и инжиниринга, Казахский Национальный медицинский университет имени С.Д.Асфендиярова, ул. Толе би 94, г. Алматы, Республика Казахстан, телефон: +7 777 015 19 19, e – mail: ibragimova.l@kaznmu.kz.

А. А. Кесикова, кандидат фармацевтических наук, менеджер по регистрации, ТОО Аббotts Казахстан, ул. Ходжанова 92, г. Алматы, Республика Казахстан; телефон: +7 701 717 50 25, e – mail: kesikova66@mail.ru.