Original Article

Determining The Authenticity Of Immunacea Bio Tablets With Immunomodulatory Action

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Abstract

Modern pharmaceutical practices are characterized by a variety of available dosage forms, but among all dosage forms, tablets are a dominant form.
This work is a dedicated determination of the authenticity of tablets developed based on raw materials of the medicinal plant Echinacea purpurea with immunomodulatory action. The purposeful preparation of drugs with immunomodulatory effects that are capable of nonspecifically stimulating the immune system and increasing the body's resistance to any pathogens is of particular importance.
In this work, the optimal composition of excipients was selected to provide necessary quality indicators for the developed tablets; the quality of the proposed and selected composition of tablets based on dried E. purpurea juice was determined.
The studied tablets called "Immunacea bio" are round, flat-cylindrical, and light brown in colour with patches and smell of vanillin. In terms of appearance, average weight and deviations from the average weight, disintegration, authenticity and microbiological purity, these tablets comply with regulatory documents. This article developed a method for determining the authenticity of active ingredients and excipients by thin-layer chromatography and determined optimal chromatography conditions, and developers of the active ingredients and excipients.

Keywords: tablets, appearance, average weight, deviations from average weight, disintegration, identity determinations and microbiological purity, thin layer chromatography, hydrophilic and hydrophobic substances.

Introduction. Modern pharmaceutical practices are characterized by a variety of dosage forms, but among all dosage forms, tablets are notable. Biomedical, production and operational advantages such as high compactness, portability, resistance to adverse mechanical and climatic factors, ease of transportation, ease and safety in handling, and long shelf life make this dosage form very popular.

Currently, in terms of morbidity of the population, a significant proportion of this morbidity is occupied by environmentally conditioned diseases, the main pathogenetic link of which is disorders of the body's immune system. In this regard, active research is underway in terms of finding new immunostimulating and immunomodulatory drugs, but most of these drugs are individual chemicals that often grossly interfere with the immune system and exhibit a number of side effects. A targeted search for medicinal plants with immunomodulatory effects that are capable of nonspecifically stimulating the immune system to increase the body's resistance to any pathogens is of particular interest (1,2).

Recently, research related to the introduction of herbal medicines into medical practice has been increasing, and the study of their chemical composition and the development of optimal technologies for obtaining affordable medicines based on these materials have also been studied. Raw plant materials are environmentally friendly, and their use is based on a deep relationship between the human body and natural components. Substances that stimulate the body's nonspecific defences must be effective, accessible, and harmless and have a convenient application scheme. These requirements are met to a certain extent by immunomodulators of plant origin, such as preparations from plants of the genus Echinacea.

The medicinal properties of Echinacea purpurea are due to the unique chemical composition of all parts of the plant (3,4,5). Echinacea is rich in essential oils, antioxidants, and essential organic acids and contains vitamins A, C and E.

In addition to vitamins, there are trace elements in the leaves, flowers and roots of E. purpurea (6), such as iron, calcium, selenium, and silicon. This composition of microelements makes it possible for preparations from Echinacea to participate in haematopoiesis and the formation of bones, teeth and nail plates, as well as hair. The microelement selenium has been included in almost all biologically active additives (BAAs) as a powerful antioxidant. Together with vitamins C and E, selenium binds free radicals and removes them from the body. Therefore, early cell ageing is prevented, as is the development of malignant neoplasms. This vitamin and mineral composition of E. purpurea determines its anti-inflammatory, antiallergy, and antimicrobial properties. Polysaccharides, contained in large quantities in the roots of E. purpurea, have an immunostimulatory property, activate the production of interferons and help damaged tissues recover faster.

The purpose of this work is to identify the authenticity of Immunacea bio tablets prepared from E. purpurea juice with immunomodulatory action.

Materials and methods. The object of the study was tablets with immunomodulatory effects.
The composition per "Immunamig SD" tablet was as follows:

Active substances:
E. purpurea juice, dried, 80 mg.
Excipients:
Aerosil, 5 mg;
Lactose monohydrate, 153.5 mg;
Magnesium stearate, 8 mg;
Saccharin sodium, 0.875 mg;
Vanillin, 1.25 mg; and
Flavour additives, 1,375 mg.
Each tablet weighed 250 mg.
The description of the tablets was carried out visually, and disintegration was determined according to the pharmacopoeia.
When determining the authenticity of the tablets, the hydrophilic substances were extracted with ethyl alcohol. The separation of the components was carried out by thin layer chromatography (TLC). The stationary phase was silica gel 60 (Merck 20*20), and the eluent was ethyl acetate:n-hexane:glacial acetic acid (50:50:1). The standard solution was applied at a volume of 10 µl, the test solutions were applied at a mass of 20 µg, and the development time was 25 minutes.

Results and discussions. The studied tablets "Immunacea bio" are round, flat-cylindrical, and light brown in colour with inclusions and smell of vanillin.
The experimental determination of some indicators of Immunacea bio tablets with immunomodulatory effects are shown in Table 1.

Table 1 The results of the study of indicators of "Immunacea bio" tablets

№	Defined indicator	Research methods	Results
1.	Description	Visually, GPh XI	Round flat-cylindrical tablets, light brown with patches, with the smell of vanillin
2.	Average weight and deviations from the average weight	GPh XI	237.5 to 262.5 mg; Deviations from the average weight + 5%
3.	Disintegration	GPh XI	At least 30 minutes

4.	Microbiological purity	GPh XI and changes No. 2	Category 3B

To determine the hydrophilic and hydrophobic substances in the tablet and to prove the authenticity of Immunacea bio, several tablets were carefully crushed and ground in a mortar until a powder was formed.

From powdered tablets, hydrophilic substances were extracted with ethyl alcohol. The separation of the components extracted with ethyl alcohol was carried out by TLC using a silica gel plate as the stationary phase. As developers, a mixture of diphenylboryloxyethylamine solution 1% solution and 5% Macrogol reagent solution was used, followed by evaluation at a length of 366 nm.

A 1% solution of diphenylboryloxyethylamine was prepared by dissolving 5 g of diphenylboryloxyethylamine in 250 ml of acetone followed by the addition of 250 ml of n-hexane. To prepare 5% Macrogol reagent, 25 ml of polyethylene glycol 400 was diluted in 250 ml of acetone, and 250 ml of n-hexane was added.

For comparison and identification of active substances, a standard sample was prepared from 3 mg of chlorogenic acid and 3 mg of caffeic acid in 10 ml of methanol. When preparing a solution from dried Echinacea juice, 10 ml of 20% ethyl alcohol was added to 0.16 g of dried juice and stirred for 20 minutes; after the specified time, the solution was filtered through a membrane filter.

To prepare a drug solution, 10 ml of 20% ethyl alcohol was added to 0.51 g of powdered tablets and thoroughly mixed; after 20 minutes, the finished solution was filtered through a membrane filter.

The chromatographic data of the standard sample and test solutions are detailed in Table 2.

Table 2 Chromatographic data of the standard sample and test solutions (hydrophilic substances)

№	Determined solution	Results
1.	Standard solution	Blue fluorescent zone with $R_{f\ value}$ – 0.25 chlorogenic acid Blue fluorescent zone with $R_{f\ value}$ – 0.80 caffeic acid
2.	Dried juice test solution	Blue fluorescent zone near the chlorogenic zone, acids on the chromatogram of a standard solution Two blue fluorescent zones in the middle and upper thirds of the chromatogram Other blue fluorescent areas may be present
3.	Test solution of Immunacea bio tablets	Blue fluorescent zone near the zone of chlorogenic acid in the chromatogram of the standard solution Two blue fluorescent zones in the middle and upper thirds of the chromatogram Other blue fluorescent areas may be present

The active substances were extracted from ground tablets with ethyl alcohol. Lipophilic substances were extracted from this solution by liquid–liquid extraction using chloroform. Separation of the components extracted with chloroform was carried out by TLC using a silica gel plate as the stationary phase. An ethyl acetate:n-hexane:glacial acetic acid (50:50:1) solution was used as the eluent; anise aldehyde reagent was used as a developer. To prepare a developing reagent, 87 ml of glacial acetic acid, 450 ml of methanol and 15 ml of sulfuric acid were added to 1.5 ml of anisaldehyde.

For the manifestation of lipophilic substances, a standard solution was prepared by dissolving 3 mg of resorcinol and 3 mg of menthol in 10 ml of menthol.

The test solutions were prepared as follows: 10 ml of 20% ethyl alcohol was added to 0.64 g of dried echinacea juice, stirred for 20 minutes, and then centrifuged for 10 minutes at a speed of 3000 rpm, and the supernatant was placed in a separating funnel. A few millilitres of water was added to the funnel and extracted twice with 20 ml of chloroform. The extract was passed through an organic phase containing sodium sulfate and evaporated to dryness. The precipitate was dissolved in 0.5 ml of absolute ethanol and filtered through a membrane filter.

Immunacea bio tablets were crushed into powder and processed further as in the above method for the proposed dried Echinacea juice.

After drying and removing all solvents, the plate was immersed in anisaldehyde. For the development of coloured zones, the plate is heated to 105 °C. The assessment is carried out in daylight.

Chromatographic data of the standard sample and test solutions are given in Table 3.

Table 3 Chromatographic data of the standard sample and test solutions (lipophilic substances)

№	Determined solution	Results
1	Standard solution	Saturated reddish-yellow zone with $R_{f\ value}$ - 0.55 resorcinol Saturated blue–violet zone R_f - 0.80 menthol
2.	Dried juice test solution	Brown area on the application line Several brown or purple areas may be present immediately above the start line. In the lower third part of the chromatogram, there is a saturated blue- violet zone Blue zone resorcinan standard solution chromatogram Blue–violet zone in the range of $R_{f\ value}$ menthol zones on the standard solution chromatogram There may be several purple zones on the front line Several other zones may be present
3.	Test solution of Immunacea bio tablets	Brown area on the application line Several brown or purple areas may be present immediately above the start line. In the lower third part of the chromatogram, there is a saturated blue- violet zone Blue zone resorcinan standard solution chromatogram Blue–violet zone in the range of $R_{f\ value}$ menthol zones on the standard solution chromatogram There may be several purple zones on the front line Several other zones may be present there is an additional greenish -yellow to brown zone with the $R_{f\ value\ of}$ resorcinol in the chromatogram of the standard solution, which appears as a result of the presence of vanillin a (cherry flavouring)

Conclusion. The composition of "Immunacea bio" tablets was established with immunomodulatory action. Such indicators of tablets included the appearance, average weight and deviations from the average weight, disintegration, determination of authenticity, and the microbiological purity of the tablets and were determined.
A technique for determining authenticity by TLC has been developed, chromatography conditions have been selected, and optimal developers of the active ingredients and excipients have been determined.

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