RESEARCH ON THE COMPOSITION DEVELOPMENT OF SUPPOSITORIES WITH ECHINACEA AND STUDY OF THEIR STABILITY

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The purpose of the work was to experimentally substantiate the composition and study the stability of extemporary suppositories with Echinacea, intended for use in complex therapy and prevention of genitourinary diseases.

Materials and methods. The object of the study was samples of suppositories made by pouring using liquid Echinacea extract as an active pharmaceutical ingredient. Cocoa butter, Witepsol H15 and Witepsol W35 were used as suppository bases. The study was carried out using modern physicochemical, pharmaco-technological and microbiological methods.

Results. On the basis of the carried out physicochemical and pharmaco-technological studies, it was found that in the manufacture of suppositories using liquid Echinacea extract, Cocoa butter or Witepsol can be used as a suppository base. The main indicators of the quality of suppository samples were also studied: organoleptic characteristics, average weight, melting point, time of complete deformation, microbiological purity, meeting the requirements of the State Pharmacopoeia.

Conclusions. As a result of the carried out physicochemical, pharmaco-technological and microbiological studies, a suppository base was selected for the creation of extemporal suppositories for the treatment and prevention of genitourinary diseases, the main quality indicators were established that meet the requirements of the State Pharmacopoeia of Ukraine, and the shelf life of the suppositories is 10 days.

Keywords. Suppositories; suppository base; melting temperature; full deformation time; solidification temperature; microbiological purity.

Introduction. With the development of scientific and technological progress, mankind has found many benefits in life. At the same time, people exposed themselves to the influence of a huge number of harmful factors (industrial waste, smog, environmental pollution, a huge amount of drugs and household chemicals).

As a result, an unprecedented spread of allergies, cancer, cases of physiological, harmful to the body, increased immunological discoordination occurred [1].

For this reason, interest in immunomodulatory drugs has increased markedly. Along with immunomodulators of bacterial, animal and synthetic origin, herbal preparations and, above all, on the basis of the herb Echinacea purpurea, occupy a worthy place in the treatment of diseases accompanied by a decrease in the body's defenses [2].

All herbal immunomodulators derived from Echinacea have general pharmacological effects such as immunostimulating, anti-inflammatory and antiviral and are used for various pathological conditions associated with a deficiency of the immune system. Echinacea preparations are used internally for infectious and septic diseases, externally – for abscesses, infected wounds, I and III degree burns and severe bedsores. There is evidence of the effectiveness of Echinacea preparations for urogenital infections and with prolonged use of antibiotics [3].

However, the range of Echinacea preparations currently available on the pharmaceutical market does not make it possible to completely solve the issue of local therapy of urogenital infectious and inflammatory diseases.

Therefore, the development of new preparations of Echinacea for use in gynecological and urological practice in the form of suppositories continues to be a promising area of pharmaceutical technology.

Currently, Echinacea preparations are available in dosage forms for enteral (capsules and powders for oral administration, tablets, oral drops, syrup) and parenteral (homeopathic injection solutions) use.

Research was carried out on the development of domestic dosage forms of Echinacea Andreeva I.N. (2000), Kurkin V.A. (2009). However, suppositories based on extracts of the herb Echinacea purpurea have not been studied until now [4, 5].

The development of new drugs of Echinacea for application in gynecological and urological practice will expand the indications for use and...
increase the effectiveness of treatment and urogenital pathologies.

The purpose of work is experimentally to substantiate the composition and study stability of extemporaneous suppositories with Echinacea, intended for use in complex therapy and for the prevention of urogenital diseases.

Materials and methods of research.

Organoleptic control was performed visually, assessing the shape, color, size, homogeneity of the suppository mass (absence in the longitudinal section of inclusions, sequins or pieces of the base).

The average weight of the suppository was determined according to SPHУ [6] by weighing 20 suppositories with an accuracy of 0.01 g. Deviations in weight should not exceed ± 5% and only two suppositories can have a deviation of ± 7.5%.

Determination of the melting temperature was performed by the open capillary method (SPHУ 2.0, 2.2.15, p. 63) [6].

Determination of the time of complete deformation was performed according to the method described in SPHУ 2.0, 2.9.22, p. 446 [7].

The solidification temperature of the base was determined by the following reason: the molten base was poured into the device until completely filled at 50 °C. The device was closed with a stopper, through which passes a thermometer with a distribution of 0.1 °C, set so that the mercury ball was approximately in the middle of the mass of the base. The device was placed in a vessel with water having a constant temperature. The molten base was stirred by light periodic shaking until turbidity appeared, after which the base was allowed to cool without stirring.

Thermometer readings were recorded every minute. The pour point was considered the temperature at which the base maintained the state of solidification for some time.

Tests for microbiological purity were performed according to SPHУ 2.3, paragraph 5.1.4.

Results of research. From the point of view of physical and colloidal chemistry, suppositories are considered as dispersed systems consisting of a dispersion medium, represented by a base, and a dispersed phase, in the role of which drugs act. Depending on the properties of medicinal substances, suppositories can create various disperse systems. Homogeneous systems are formed when the medicinal substance is dissolved in the base. Heterogeneous systems are formed when medicinal substances are introduced into the base as an emulsion or suspension.

In the structure of suppositories, as in other dosage forms, the main (medicinal substances) and auxiliary components are distinguished. The largest part of the excipients (more than 90%) in suppositories, as well as in the suppository itself, is a suppository base.

The therapeutic effect of suppositories is carried out due to the complex action of drugs and a suppository base, which provides optimal structural-mechanical and corresponding rheological properties and is one of the most important characteristics that determine the stability of bound-dispersed systems.

The base, which makes up most of the suppository, has certain physicochemical properties and significantly affects the bioavailability of drugs, therapeutic effect, uniformity of distribution of ingredients, dosing accuracy, etc. [8].

According to the definition of the European Pharmacopoeia, suppositories contain one or few active substances dispersed or dissolved in a simple or complex base, which can be dissolved or dispersed with water or melted at body temperature.

In the international pharmaceutical market, suppository bases of various kinds are presented under well-known brands, which differ in the length of the hydrocarbon chain of saturated acids, the ratio of glycerides, and, therefore, are characterized by certain qualities. When conducting technological research and preparation of drugs, it is important to know the characteristics of each suppository base.

Requirements for suppository bases can be divide into 2 groups: biopharmaceutical and technological.

Requirements for suppository bases, justified from the biopharmaceutical point of view: the melting or dissolving temperature of the base should be close to human body temperature and not higher than 37 °C; the base should be physiologically indifferent (not to irritate mucous membranes and not to cause other undesirable effects); the base must be chemically indifferent (do not interact with drugs that are introduced into the base); the base should not interfere with the release and therapeutic effect of drugs [9].

According to technological requirements, the base must: to provide chemical and physical stability in the process of manufacturing and storage of suppositories; have the ability to easily form and maintain the required hardness when introduced; have the ability to emulsify the required amount of aqueous solutions; have certain structural and mechanical criteria of
plasticity, viscosity, deformation, etc.; have a clear melting point in a small temperature range without a softening stage; harden quickly, be technological, easy to form, pour, press [8].

In suppositories, the active substances and substances of the base form a single whole. All components of suppositories affect the effectiveness of this dosage form when administered rectally. Any changes in the chemical and physical properties of the components affect the specified unity. For this reason, there is no universal suppository mass.

Only the correct selection of the base, taking into account its personal qualities and interaction with the active substance allows you to achieve the maximum effect from suppositories.

Intravaginal route of administration of drugs is used to localize the pathological process. Rectal suppositories are used to achieve a local or general therapeutic effect [10]. In our studies, suppositories for rectal use were prepared based on liquid Echinacea extract.

The first stage of technological research is the selection of the optimal base for suppositories, capable of including liquid components without prior thickening. The dosage was chosen based on a single dose recommended for extract of Echinacea – 15 drops per dose [11, 12].

The quality of suppositories, their therapeutic effectiveness largely depend on the properties of suppository bases.

In our work we used the following lipophilic bases: cocoa butter, Witepsol H 15, Witepsol W 35 and hydrophilic polyethylene oxide (PEO) base composition: PEO 1500 ~ 95%, PEO 400 ~ 5%.

These bases were chosen because they are widely used in pharmaceutical production, and cocoa butter, which is not used in its pure form in the factory, was taken as a classic suppository base. In addition, because cocoa butter is widely used in extemporaneous formulas.

Physicochemical and structural-mechanical parameters recommended by SPbU were determined for the listed bases.

From table 1 it follows that all selected bases meet the requirements of the normative documentation in terms of melting point.

An important technological indicator is also the solidification temperature of the base. The results of the determination are presented in table 2.

### Table 1.

<table>
<thead>
<tr>
<th>Suppository base</th>
<th>Melting point, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Requirements of ND</td>
</tr>
<tr>
<td>Cacao butter</td>
<td>30.0-34.0</td>
</tr>
<tr>
<td>Witepsol H15</td>
<td>33.5-35.5</td>
</tr>
<tr>
<td>Witepsol W35</td>
<td>33.5-35.5</td>
</tr>
</tbody>
</table>

### Table 2.

<table>
<thead>
<tr>
<th>Suppository base</th>
<th>Solidification temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cacao butter</td>
<td>26.3±0.2</td>
</tr>
<tr>
<td>Witepsol H15</td>
<td>32.3±0.5</td>
</tr>
<tr>
<td>Witepsol W35</td>
<td>31.2±0.4</td>
</tr>
</tbody>
</table>

As follows from the data, the solidification temperature is lower than the melting temperature, which will make it possible to obtain suppositories by casting with a favorable temperature regime.

**Establishing a substitution factor for the studied bases**

When preparing suppositories by pouring in order to accurately dose the drugs should be pre-established substitution factor for the drug in relation to each of the bases and considering it to calculate the required amount of base.

The accuracy of dosing in the method of pouring depends on the size of the deepening of the form, the density of the base and the drugs that are part of the suppositories, the uniformity of mixing the base with the active substance.

The substitution factor is not constant, but varies depending on the viscosity of the base, the particle size of the drug substance, the density and amount of drug substances.

The substitution factor was calculated by the formula:

\[ F = \frac{P - O}{A} + 1, \]

where: \( P \) – weight of 30 suppositories without drug substance, g;

\( O \) – weight of 30 suppositories with the drug substance, g;
A – the total weight of the drug in 30 suppositories, g;
F – substitution factor.
The calculation of the amount of base taking into account the substitution factor was performed according to the formula:

\[ J = (P - F \times A) \times n, \]

where: P – the average weight of the suppository without the drug substance, g;
A – the amount of drug substance for 1 suppository, g;
F – substitution factor;
n – the number of suppositories for which the calculation is made.

Loading amounts of different bases with Echinacea extract, taking into account the calculated substitution factors are given in table 3.

As the results show, the substitution factors of Echinacea extract for lipophilic bases are approximately the same, and their values are in the range of 0.918-0.932.

Table 3. Factors substitution and quantities of the base required for the preparation of suppositories with Echinacea extract

<table>
<thead>
<tr>
<th>Type of base</th>
<th>Weight of suppository without drug substance, g (P)</th>
<th>Mass of suppository with medicinal substances, g (O)</th>
<th>Substitution factor (F)</th>
<th>Number of suppositories for which the calculation is made (N)</th>
<th>Calculation of bases taking into account the substitution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cacao butter</td>
<td>2.237</td>
<td>2.278</td>
<td>0.932</td>
<td>30</td>
<td>50.33</td>
</tr>
<tr>
<td>Witepsol H15</td>
<td>2.237</td>
<td>2.286</td>
<td>0.918</td>
<td>30</td>
<td>50.67</td>
</tr>
<tr>
<td>Witepsol W35</td>
<td>2.244</td>
<td>2.289</td>
<td>0.925</td>
<td>30</td>
<td>50.59</td>
</tr>
</tbody>
</table>

Suppositories were prepared by pouring with a content of 30 or 40 drops of Echinacea extract. Echinacea extract was introduced into suppository bases by type of emulsion.

For the manufacture of suppositories with Echinacea extract by pouring used detachable forms that allow to obtain suppositories weighing 1.22-1.25 or 2.24-2.26 g. Suppositories were prepared without the use of an emulsifier, as the base has emulsifying properties. The suppositories had a smooth surface, the same torpedo-shaped, homogeneous mass without inclusions.

Homogeneity of suppositories was determined visually by the absence of sequins, inclusions and pieces of the base on the longitudinal section.

The average weight was determined by the method of SPhU. The obtained results testify to the compliance of the prepared suppositories with the requirements of the normative documentation in terms of deviation from the average weight.

Structural and mechanical properties of suppositories were evaluated by the following indicators: melting point, solidification temperature, time of complete deformation.

The results of determining the structural and mechanical properties are given in table 4.

Table 4. Structural and mechanical indicators of suppositories with Echinacea extract

<table>
<thead>
<tr>
<th>Type of base</th>
<th>Temperature, °C</th>
<th>Time of full deformation, sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cacao butter</td>
<td>34.2</td>
<td>27.8</td>
</tr>
<tr>
<td>Witepsol H15</td>
<td>36.4</td>
<td>32.1</td>
</tr>
<tr>
<td>Witepsol W35</td>
<td>36.7</td>
<td>31.5</td>
</tr>
</tbody>
</table>

The obtained data indicate that suppositories with Echinacea extract meet the requirements of the normative documentation, i.e., the introduction of liquid extract does not have a significant effect on the physicochemical and structural-mechanical properties of suppository bases.

Significant influence on the manifestation of pharmacological activity of medicinal substances in the composition of various drugs has the right choice of excipients that increase or decrease the rate of release of active substances, as well as improve the quality of some technological operations.

The presence of surfactants in the base has a significant effect on the completeness of the release of active substances from suppositories [13].

Since suppositories with Echinacea extract are developed for the purpose of their production...
in pharmacies, the introduction of surfactants is not required.

As a result of research, the following structure of new extemopaneous suppositories with a liquid extract of an Echinacea was established:

Echinacea extract liquid 0.8 g
Witepsol or cocoa butter is enough to obtain suppositories weighing 2.5 g

The evaluation of the quality of suppositories was carried out in accordance with the requirements of the SPhU for this dosage form.

Appearance: suppositories cigar-shaped or torpedo-shaped, of the same size, with a smooth surface, sufficient hardness to ensure ease of administration, dark cream in color. The absence of inclusions, determined visually on the cut, indicates the homogeneity of the suppositories.

The average weight of suppositories was 2.2 ± 0.05 g, the deviation in weight was within ± 5%.

The melting temperature for suppositories is 36.4 ± 0.5 °C, which meets the requirements of regulatory documents.

The time of complete deformation for suppositories is 7.0 ± 1.0 minutes, which meets the requirements of the SPhU [14].

Currently, there are certain requirements for the microbiological purity of suppositories:
- total number of aerobic bacteria – no more than 1000 in 1 g or 1 ml;
- total number of mushrooms – no more than 100 in 1 g or 1 ml;
- absence of Escherichia coli in 1 g or 1 ml.

The tests were carried out immediately after the preparation of the suppositories and after 14 days, since the suppositories are extemoporal.

To test suppositories for microbiological purity, a 10 g sample of suppositories under aseptic conditions was emulsified in 100 ml of phosphate buffer solution pH 7.0 (PBS) using glass beads and a minimum amount of emulsifier Tween-80; in this case, mechanical shaking and heating were used to a temperature not exceeding 45 °C.

After obtaining a homogeneous emulsion, the sample was diluted in 10 ml of sterile PBS to 1: 100 and 1: 1000, then 1 ml of each dilution was introduced into sterile Petri dishes, followed by pouring onto medium No. 1 (nutritious meat-peptone agar) and No. 2 (Sabour’s medium). Petri dishes with the solidified medium were inverted and incubated at 32.4 °C and 22.5 °C. After 48 hours and finally after 5 days, the number of grown colonies of aerobic bacteria and fungi was counted.

The final number of bacterial colonies was counted from two dishes, the average value was found, and multiplied by the corresponding dilution.

When testing for Escherichia coli, samples of suppositories in an amount of 10 g were placed in medium No. 11 (lactose broth), emulsified using glass beads and Tween-80 and incubated for 3 hours at a temperature of 32.5 °C. Then 10 ml of inoculated medium No. 11 was placed in 100 ml of medium No. 3 (medium for enrichment of the family Enterobacteriacea) and incubated at 32.5 °C for 18-24 h [15].

After incubation, a bacterial loop was inoculated onto medium No. 4 (Endo agar). On Endo’s medium, colonies of Escherichia coli form characteristic crimson colonies 2 mm in diameter with or without a metallic sheen. If there is no growth of colonies, a negative result is given about the presence of Escherichia coli in the sample. The results are shown in Table 5.

In terms of microbiological purity, suppositories meet the requirements – the total number of aerobic bacteria and fungi (in total) in 1 g of the drug did not exceed 10². Enterobacteriaceae, staphylococcus and Pseudomonas aeruginosa were not found in suppositories.

In table 6 the results are given for all parameters that were checked during storage in order to confirm the stability of the suppositories.

Table 5. Results of a microbiological study of suppositories with Echinacea extract

<table>
<thead>
<tr>
<th>Results</th>
<th>Determination of the number of aerobic bacteria in 1 g of sample</th>
<th>Determination of the total number of fungus in 1 g of sample</th>
<th>Determination of E. coli in 1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately after preparation</td>
<td>1.65*10²</td>
<td>0.2*10¹</td>
<td>negatively</td>
</tr>
<tr>
<td>After 14 days</td>
<td>2.01*10²</td>
<td>0.3*10¹</td>
<td>negatively</td>
</tr>
<tr>
<td>Dilution fluid control</td>
<td>When sowing a diluting liquid (PBS with tween) on a nutrient medium, the growth of microorganisms was not detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium control</td>
<td>When culture media were incubated without inoculation in a thermostat at t = 32.5 °C during all studies, no growth of culture media was recorded</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.

Quality indicators of suppositories with Echinacea extract during natural storage at a temperature of 4 ± 1 °C

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Standardized requirements</th>
<th>Shelf life, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Homogeneous cream color with a yellowish tinge without mechanical impurities and glitter</td>
<td>Homogeneous cream color with a yellowish tinge without mechanical impurities and glitter</td>
</tr>
<tr>
<td></td>
<td>Homogeneous cream color with a yellowish tinge without mechanical impurities and glitter</td>
<td></td>
</tr>
<tr>
<td>Average weight, g</td>
<td>from 2,17 till 2,25</td>
<td>2,2±0,05</td>
</tr>
<tr>
<td>Melting point, °C</td>
<td>not higher than 37 °C</td>
<td>36,4±0,5</td>
</tr>
<tr>
<td>Full deformation time, min</td>
<td>no more than 15</td>
<td>7,0±1,0</td>
</tr>
</tbody>
</table>

As follows from the data, the prototypes of suppositories with Echinacea extract had quality indicators that meet the regulatory requirements during the observed period of 14 days, therefore, the shelf life is 10 days.

Conclusions

1. Based on the obtained research results, a preliminary composition of new extemporaneous suppositories with Echinacea extract as an immunomodulatory agent was developed.

2. The research of organoleptic indicators of samples of the developed suppositories is carried out and the rational suppository basis is chosen.

3. As a result of the conducted researches the technology of preparation of immunomodulatory suppositories on the basis of a liquid extract of Echinacea purpurea is developed.

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В якості носіїв використовували масло какао, вітепсол H15 та вітепсол W35. Дослідження проводили із використанням сучасних фізико-хімічних, фармако-технологічних та мікробіологічних методів.

Результати. На підставі проведених фізико-хімічних та фармако-технологічних досліджень було встановлено, що при виготовленні супозиторіїв із використанням рідкого екстракту екінзії можливо застосовувати в якості носіїв масло какао або вітепсол. Також, було виведено основні показники якості зразків супозиторіїв: органолептичні характеристики, середню масу, температуру плавлення, час повної деформації, мікробіологічну чистоту, які відповідають вимогам ДФУ.

Висновки. В результаті проведених фізико-хімічних, фармако-технологічних та мікробіологічних досліджень було обрано супозиторну основу при створенні екстемпоральних супозиторіїв для лікування та профілактики сексуальних захворювань, встановлено основні показники якості, які відповідають вимогам ДФУ, та термін зберігання лікарського препарату 10 діб.

Ключові слова. Супозиторії; супозиторна основа; температура плавлення; час повної деформації; температура затвердіння; мікробіологічна чистота.

Conflicts of interest: authors have no conflict of interest to declare.

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A – концепція та дизайн дослідження; B – збір даних; C – аналіз та інтерпретація даних; D – написання статті; E – редагування статті; F – остаточне затвердження статті.

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