

Development of the composition and manufacturing technology of the new combined drug Lavaflam

INTRODUCTION

Treatment of diseases of the biliary system is one of the urgent problems of modern medicine (1-4). Diseases of the liver and biliary tract are very common diseases of internal organs and occur in 10-20% of the population of developed countries. According to statistics, in Ukraine, the number of such patients is constantly increasing, in 10 years the prevalence of increase is 97% (5).

Currently, the combined drugs are leading among pharmacotherapeutic agents, including the treatment of diseases of the biliary system, which require complex treatment. Choice of drug combination allows to expand the range of action of the drug and the complex influence on the disease, enhance the activity of the every ingredient, as well to improve tolerability and reduce side effects (6, 7).

Modern herbal medicines are widely used in complex treatment of various diseases, including biliary system. They are characterized by high efficiency, low toxicity and the possibility of long-term use without the risk of side effects (5, 8).

Complex treatment of billiary treatment is the reason for the creation drugs of herbal origin, because herbal remedies usually posses a lot of pharmacological effect and have low level of side effects. Nowadays it is widely used immortelle-containing drugs which are well-known in Ukraine and some countries of former USSR. One of them is Flamin with choleric action, but as well established hepatoprotective, antimicrobial and antivirus action. That why it is possible to enlarge the variety of action by combination flamin with lavender oil. Adding lavender oil to flamin increases peristalsis of billiary tract, improves detoxication function of liver (8-11). Pre-clinical research in vivo was conducted under the direction of prof. Drogozov S.M. in the department of pharmacology of National University of Pharmacy, Kharkov, Ukraine and confirmed the indicated effects (9).

The aim of research was development of composition and technology of the original drug in the form of tablets, which include a combination of herbal substances flamin and lavender oil.

MATERIALS AND METHODS

The objects of research was medicinal substances of herbal origin flamin (0.05 g), lavender oil (0.02 g), as well as excipient substances and tablets named «Lavaflam».

Lavaflam is new drug in the form of tablets, consist from flamin, lavender oil and excipients: β -cyclodextrin (0.27 g), mannitol PARTECK M 200 (0.20 g), croscarmellose sodium (0.03 g), potato starch (0.022 g), PEG 6000 (0.002 g), magnesium stearate (0.006 g).

To achieve this goal it was necessary to carry out the analysis of biological active compounds of flamin and lavender oil, their stability in tablets, as well to make choice of excipients and to carry out pharmacotechnological analysis of them.

Flamin (Pharmaceutical company «Zdorovyе», Kharkov, Ukraine) - yellow or with brownish yellow touch of color powder with a weak specific smell, easily soluble in 96% alcohol, practically insoluble in water and chloroform (12). Flamin is obtained from the Immortelle flowers (*Helichrysum arenarium* (L.) Moench, Asteraceae) by extraction of 50% ethanol followed by purification (13,14). It contain flavonoid glycosides and aglycones (salipurposide, isosalipurposide, kaempferol, luteolin, naringenin, apigenin and others), essential oils, organic acids, polysaccharides and other biologically active substances of different groups (13-15). Antimicrobial and antiviral activity of the flamin and other drug from immortelle flowers has been established (6, 10, 13, 14, 17-21).

Analysis of total flavonoids were carried using spectrophotometry with the reference into isosalipurposide-standard (specific absorbtion index (7, 13, 14, 16).

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Assay of total flavonoids in Flamin

The test solution. About 0.60 g (accurately weighed) powdered tablets are placed in a 100 ml volumetric flask, add 70 ml of 96% ethanol, allow to stand in an ultrasonic bath for 5 minutes, dilute to 100 ml with the same solvents, stirred and filtered through a paper filter «blue ribbon», discarding the first 15 ml of filtrate. 2.0 ml of the resulting solution is diluted with 96% alcohol to a volume of 50.0 ml and stirred.

Measure the absorbance of the test solution at a spectrophotometer (UV-VIS HP 8453, company «Hewlett Packard» (USA) at a wavelength 315 nm in a cuvette with a 10 mm - thickness, using 96% alcohol as a compensation solution.

Calculate the total flavonoid expressed as isosalipurposide ($X_1, \%$), in a single tablet, using the Equation 1:

$$X_1 = \frac{A \times 100 \times 50 \times b}{E_{1sm}^{1\%} \times m \times 2 \times 100} = \frac{A \times 25 \times b}{E_{1sm}^{1\%} \times m}, \quad (\text{Equation 1})$$

where: A - the absorbance of the test solution at $\lambda=315$ nm;

$E_{1sm}^{1\%}$ - isosalipurposide's specific absorption (96% alcohol) at $\lambda=315$ nm is 260;

m - mass of the drug sample, in grams;

b - the average weight of the tablets, in milligrams.

Amount of total flavonoids expressed as isosalipurposide should be 31.0-39.0 mg of the nominal content.

Lavender oil is obtained from flower of *Lavandula angustifolia* Mill, Lamiaceae. Lavender oil (Pharmaceutical company «Zdorovyie», Kharkov, Ukraine) is a transparent, colorless or pale yellow liquid with a specific fragrance (22, 23). Analysis of lavender oil was carried out according to the requirement of European Pharmacopoeia and Ukrainian State Pharmacopoeia, which regulate the content of the following main components in lavender oil (in %): limonene - less than 1.0; 3-octanone - 0.1-2.5; camphor - less than 1.2; linalool - 20.0-45.0; linalyl acetate 25.0-46.0; terpinene-4-ol - 0.1-6.0; lavandulyl acetate - more than 0.2; lavandulol - more than 0.1; α -terpineol - less than 2.0. From the above normalization it implies that the main components of lavender oil are linalool and linalyl acetate. Analysis of these terpenoids was carried out using gas chromatography (GC) using an internal normalization. A method for the assay of lavender oil in a combined pharmaceutical preparation «Lavaflam» were developed using a gas chromatograph Agilent 7890 (USA) with a flame ionization detector. The lavender oil was used (1 mg / ml) as the standard sample solution. The calculation was performed for the amount of linalool and linalyl acetate (11).

Methods of analysis of lavender oil in «Lavaflam» tablets

The test solution. About 0.75 g (accurately weighed) of 20 powdered tablets were placed in a 25 mL volumetric flask, add 15 mL of methanol, allow to stand in an ultrasonic bath for 10 minutes, dilute the volume with the same solvent and thoroughly mixed. The resulting suspension is filtered through a paper filter «blue tape», discarding first 5 mL of filtrate. The solution is used freshly prepared.

Reference solution. About 0.5 g (accurately weighed) of lavender oil is placed in 50 ml volumetric flask, dilute with 50 ml methanol and thoroughly mixed. 5.0 ml of this solution is placed in a 50 ml volumetric flask, dilute with methanol and thoroughly mixed. The solution is used freshly prepared.

1 μ l of test solution and reference solution were chromatographed using a gas chromatography with a flame ionization detector.

Lavender oil content (X_2) in grams per tablet was calculated using the Equation 2:

$$X_2 = \frac{\sum S_i \times m_o \times 5 \times 25 \times b}{\sum S_o \times m \times 50 \times 50} = \frac{\sum S_i \times m_o \times b}{\sum S_o \times m \times 20}, \quad (\text{Equation 2})$$

where: ΣSi - average value of the sum of areas of peaks of linalol and linalyl acetate, calculated from the chromatogram of the test solution;

ΣSo - average sum of the areas of peaks of linalol and linalyl acetate, calculated from the chromatogram of the reference solution;

b - the average tablet weight calculated for 20 tablets; in grams;

m_o - mass of lavender oil, in grams;

m - mass of sample preparation, in grams.

Normalization lavender oil content is set within 90% - 110% of the nominal content.

Pharmaco-technological studies have been carried out for development of the composition of excipients (24). The bulk density, the tap density, the flowability and the angle of repose, compressibility, friability, resistance of tablets to crushing were determined on the devices of company «Pharma Test» (Germany), the disintegration on the apparatus «Erweka» (Germany). Flowability was evaluated by the Carr Index and Hausner Index (25, 26).

Pharmacological and technological properties of powders and granulates

Determination of the Bulk Density

The bulk density is the weight of a unit volume of a powder loosely placed into a measuring cylinder. It depends on density of a substance, the particle size and shape, its compatibility. Into the dry cylinder introduce 100.0 g of the substance being examined without compacting. Calculate the bulk density in g per ml by the formula m/V_0 .

Determination of the Density

Fix the cylinder in its holder. Carry out 10, 500 and 1250 taps and read the corresponding volumes V_{10} , V_{500} and V_{1250} to the nearest millilitre. Calculate the tap density in g per ml by the formula m/V_f , in which V_f is the final tapped volume.

Determination of Flowability

Flowability characterizes the ability of a material to pour out from the container (a feeding funnel) under its own weight. Flowability was determined by outlet velocity of the fixed quantity of the material (100 g) pouring out from a metal funnel with strictly geometrical parameters and by the angle of repose. The flowability is expressed in seconds, related to 100 g of the sample.

Determination of angle of repose

The indirect characteristic of flowability is the angle of repose. After pouring out from a funnel the powdery material forms a conic hill on a horizontal plane. The angle between incline and the basis of this hill is called the angle of repose expressed in degrees. It was determined with a goniometer or measured in another way. The angle of repose changes over a wide range from 25-35° for well flowing materials and up to 60-70° for poorly flowing ones.

Determination of Carr Index and Hausner Index

Carr Index and Hausner Index are used in describing the flowability of powder. According to Carr, an excellent flowability is between the Carr Index of 5% to 15% while Carr Index of above 25% normally shows poor flowability.

$$CarrIndex = \frac{(\rho_{tap} - \rho_{bulk})}{\rho_{tap}} \cdot 100\%$$

Hausner Index of 1.0 to 1.1, powder was considered as free flowing, greater than 1.1 to 1.25, powder was classified as medium flowing, greater than 1.25 to 1.4, the powder was classified as difficult to flow and higher than 1.4, powder was considered to be very difficult to flow.

$$HausnerIndex = \frac{\rho_{tap}}{\rho_{bulk}}$$

Determination of Compressibility

To determine compressibility of a material it was compressed a sample of the powder (0.3 – 0.5 g) within a matrix of 9 or 11 mm in diameter, respectively, on hydraulic press at pressure 120 MPa. Compressibility also can be also evaluated by hardness of the obtained tablet.

Used excipients

To obtain tablets lavender oil was converted to the solid state. Lavender oil granulate prepared by solid phase method, as an excipient substance was used β -cyclodextrin.

Cyclodextrins are used in pharmaceutical technology in order to build complex systems with a variety of APhI (active pharmaceutical ingredients), thereby largely improving their bioavailability and solubility, and increased chemical and physical stability. Cyclodextrin complexes are used to mask the unpleasant taste of the active substances and converting liquid substances into solids (27-30).

Granulak 70 («Meggle Excipients», Germany) is a crystalline form of the lactose, it consists of fine particles with sharp edges and has cohesion properties that can be useful in the process of granulation.

As fillers were used the following substances: tabletoza 70, MCC 102, di-calcium phosphate (for direct compression), mannitol PARTECK M 200 (for direct compression) and saccharose compressible grade B.

To improve the disintegration were used disintegrants (30, 31). According to the literature (30) as disintegrant is better to use starch in combination with other substances with disintegrating action. On powdering step as disintegrant used various combinations of the potato starch with the following excipients: sodium croscarmellose, sodium starch glycolate and crospovidone. To prevent adhesion, improve flowability and plasticity of the granulate for tableting lubricants were used. For this purpose, dusting granulate stage was used magnesium stearate in combination with PEG 6000.

Preparation of granulate

Preparation of granulate A

In a laboratory mixer β -cyclodextrin was mixed with water (30-40%) for 3-5 minutes until a paste was formed. To the resulting paste lavender oil was added with stirring during 5-10 min. The resulting mass was dried in a tray dryer at room temperature for 48 hours, stirring periodically. After drying the mass was granulated through a sieve (holes diameter 1.0 mm) to obtain a homogeneous granulate (granulate A).

Preparation of granulate B

Considering obtained results of flowability values, it is advisable to use for powder flamin as a filler PARTECK M 200 mannitol in an amount of 0.2 g. Due to the spherical shape of granulate mannitol PARTECK M 200 is evenly distributed between the particles of flamin powder, while improving the flowability and uniformity of the resulting granulate mass. When mixed flamin with mannitol PARTECK M 200, than granulated through a sieve (holes diameter 1.0 mm) and obtained granules B.

Quality control tests of tablets

Determination of Tablets Friability

The friabilator of drum type was used to determine abrasion of tablets. Tablets are dedusted and weighed with an accuracy of 0.001 g, then they are placed in a drum, are covered with a lid and the device is switched up to 4 minutes, a drum has 100 revolutions. After that the tablets are dedusted and if they do not have chips and cracks their mass is determined with an accuracy of 0.001 g. The abrasion of tablets in percentages is calculated by the formula:

$$U = \frac{m_1 - m_2}{m_1} \cdot 100\%, \text{ where}$$

m_1 –mass of tablet before friability,
 m_2 - mass of tablet after friability

The loss in the mass of the tested tablets should be not more than 1% of the total mass of the tested tablets.

Determination Resistance of Tablets to Crushing

This test is intended to determine, under defined conditions, the resistance to crushing of tablets measured by the force needed to disrupt them by crushing. The apparatus consists of 2 jaws facing each other, one of which moves towards the other. It was placed the tablet between the jaws taking into account, where applicable, the shape, the break-mark and the inscription; for each measurement orient the tablet in the same way with respect to the direction of application of the force. I was carried out the measurement of 10 tablets.

The load that caused the destruction of the tablets is a measure of strength. Received strength value measured in Newtons

Determination of Tablets Disintegration

This test is provided to determine whether tablets disintegrate within the prescribed time when placed in a liquid (water). Place 1 dosage unit in each of the 6 tubes of the basket. Operate the apparatus using the specified medium adjusted at 37 ± 2 °C as the immersion liquid. At the end of the specified time, lift the basket from the liquid and observe the dosage units: all of the dosage units have disintegrated completely. A tablet should be broken up within not more than 15 min.

Determination of Average Weight of Tablets

Each of 20 tablets is weighed separately with an accuracy of 0.001 g and average mass of them is calculated. The deviation of the average mass of tablets from the mass specified in the "Composition" section should not exceed $\pm 5\%$.

Statistical analysis

All analysis were carried out in triplicates. The results of research were performed from average of all samples reading mean \pm standard deviation using Excel 2007 and STATISTICA 6.0.

RESULTS AND DISCUSSION

According to the literature it is known (30) that the concentration of β -cyclodextrin affects on the stability of essential oils during storage.

To determine the optimal concentration of β -cyclodextrin granules with lavender oil samples were prepared with different amount of β -cyclodextrin: 0.15 g; 0.20 g; 0.25 g; 0.27 g; 0.30 g. Stability during storage for 5 weeks keeping, was determined by the analysis of concentration of lavender oil. The main components of lavender oil are linalool and linalyl acetate. Analysis of the lavender oil's components was carried out by gas chromatography. As a

control (without β -cyclodextrin) was used to compare the stability of granules with lavender oil and granulak 70.

Effect of β -cyclodextrin concentration on lavender oil stability during storage is shown in Figure 1.

β -cyclodextrin has a stabilizing effect on the lavender oil during storage. The granulate which contains β -cyclodextrin in dose 0.30 g and 0.27 g of lavender oil concentration was not changed and a constant value was 0.02 g for 5 weeks. The granulate with granulak 70 (without β -cyclodextrin), lavender oil concentration after 2 weeks storage decreased almost 2-times. Thus on the basis of the research, we have chosen the concentration of β -cyclodextrin in a granulate with lavender oil, 0.27 g, which allows to maintain stability during storage for 5 weeks.

Results of studies of stability of the lavender oil granulate with β -cyclodextrin in an amount of 0.27 g at different temperatures (20 ± 5) °C and (40 ± 5) °C are shown in Figure 2.

It is found that at a temperature of (20 ± 5) °C lavender oil concentration value is stable for 12 months, and at a temperature of (40 ± 5) °C after 3 months storage lavender oil concentration in the granulate gradually decreases. These results allow us to determine the temperature at which lavender oil will remain stable during storage.

It has been found that the lavender oil is almost completely lost for 12 days at research of the stability of powdered mixture of lavender oil and β -cyclodextrin (obtained without the hydration process) at 20 ± 5 °C. Consequently the complex is formed only in the presence of water.

The obtained results confirm the formation of the complex due to the process of hydration and optimal concentration of β -cyclodextrin. The molecule of β -cyclodextrin has the form of truncated cone that is hollow inside. In the presence of water the inner cavity because of glycoside molecules gets hydrophobic properties and it plays the role of a "master. This arrangement allows to place "guest molecules" of lavender oil inside the cavity and thus to stabilize it. Microscopic observations showed that there are no oil inclusions of free lavender oil in the samples. t confirms the formation of the complex.

For the development of the composition of the excipients of tablets and a choice of rational technology, the next step of the research was to study the pharmaco-technological properties of the flamin (Table 1).

The results of technological properties of the substance flamin (Table 1) show that it has low flowability. This fact is confirmed by microfine and complex surface of the powder particles and the high value of the angle of repose. The difference in bulk density and tap density indicates that powder has caking ability. Indicators Hausner Index and Carra Index also showed poor flowability value. Compressibility is characterized by model tablet strength after depressurization. Polydisperse particle of flamin powder contributes strength tablets. The substance has a satisfactory value of flamin compressibility. The greater the compressibility of the powder, the higher strength tablets.

Pharmaco-technological research of powder of flamin substance showed that to improve the flowability and the production of tablets is necessary to use complex of corresponding excipients to obtain a granular mass with the flamin (31).

Next step of research was to obtain the granulated mass to its base by introducing excipient substances from the group of fillers, which improve the processability for improving technological properties of flamin (Table 2).

In order to choose optimal filler there were obtained granular mass of flamin with substances which are shown above (Table 2) and it was determined flowability of each of them. The results are shown in Figure 3.

The use of excipient substances contributes to the formation of a granular mass with the powder of flamin and improves the value of flowability. As can be seen from Figure 3, for each filler these values are different and depend on the pharmaco-technical properties of each material, which are described in Table. 2. Relatively good results are obtained for the granular mass mannitol PARTECK M 200 (30 sec/100 g sample), just below the phosphate di-calcium (33 sec/100 g sample) and sucrose in the pressing variety (35 sec/100 g of sample). For granular mass with tabletoza 70 and MCC 102 values of flowability was poor (unsatisfactory).

Results of effect of amount of mannitol PARTECK M 200, di-calcium phosphate and saccharose pressing grade B on the flowability value are shown in Figure 4.

Figure 4 shows that increase of amount of filler improves the flowability value, respectively. When adding to the powder of flamin mannitol PARTECK M 200 in an amount of 0.2 g flowability becomes satisfactory and get value of 15 sec/100 g sample. With further increase the amount of mannitol in granulated mass PARTECK M 200 flowability value is practically unchanged. At the same concentration of fillers for powder of flamin flowability value with di-calcium phosphate is 20 sec/100 g of sample, and for saccharose compressible grade B - 25 sec/100 g sample.

Considering obtained results of flowability values, it is advisable to use for powder flamin as a filler PARTECK M 200 mannitol in an amount of 0.2 g.

Next step of research was to combine and mix granular mass. In a laboratory mixer lavender oil granulate with β -cyclodextrin in an amount of 0.27 g (granulate A) was combined with flamin granulate (granulate B), stir, and then the resulting granular mass was screened through a sieve with a 1.0 mm - hole diameter.

An important indicator of the quality that affects on the bioavailability of a drug is a disintegration.

Combination of disintegration time for combination of disintegrating agents is from 10 to 18 minutes depending on the nature of disintegrants (Figure 5). Disintegration time for the starch, as opposed to a combination of substances is set higher at 18 minutes. The combination of disintegrating agents has a complex mechanism of action on the tablet disintegration due to the effect of wettability, capillarity and swelling. The result of the experiment revealed that the sodium croscarmellose in combination with potato starch has a substantial effect on the disintegration of the tablet, and ultimately on the disintegration time, which is 10 min.

Due to the results of research it was developed composition of tablet excipients for tablet «Lavaflam»: β -cyclodextrin (0.27 g), mannitol PARTECK M 200 (0.20 g), croscarmellose sodium (0.03 g), potato starch (0.022 g), PEG 6000 (0.002 g), magnesium stearate (0.006 g).

Tablets were prepared by compressing method using separate granulation technique, which consists of the following process steps:

1. Preparation of raw materials
2. Preparation of lavender oil granulate with β -cyclodextrin (granulate A)
3. Preparation of a granulate with a flamin (granulate B)
4. The mixing, sifting and dusting of granulates A and B
5. Tableting and dedusting
6. Packing of tablets in blisters
7. Packaging blisters in packs
8. Packaging packs into group container.

Standardization of «Lavaflam» tablets were performed on the following parameters: appearance, geometrical sizes, average weight, disintegration, abrasion resistance, compressive strength, assay (the amount of flavonoids with the reference into isosalipurposide, analysis of lavender oil with the reference into linalool and linalool acetate). The results are shown in Table 3.

Results of research indicated that tablets «Lavaflam» (Table 3) meets the European and Ukrainian State Pharmacopoeia requirements (Ukrainian Pharmacopoeia is harmonized with

European Pharmacopoeia). Specification for control of quality of tablets «Lavaflam» was developed (11, 16).

CONCLUSION

For a complex treatment of diseases of the biliary system it was proposed the original pharmaceutical drug in the form of combined pills «Lavaflam», which include the composition of herbal components of flamin and lavender oil. Tablets were prepared by pressing for separate granulation technology. Lavender oil granulate prepared by solid phase method, as an excipient substance was used β -cyclodextrin. It is applied as a choleric and anti-inflammatory agent in the case of cholecystitis, cholangitis and biliary dyskinesia. Flamin granulate is prepared by mixing with spherical shape filler mannitol PARTECK M 200. On the basis of studies designed composition tablet excipients «Lavaflam»: β -cyclodextrin (0.27 g), mannitol PARTECK M 200 (0.20 g), croscarmellose sodium (0.03 g), potato starch (0,022 g), PEG 6000 (0.002 g), magnesium stearate (0.006 g). Assay of the main components of lavender oil with the reference into linalol and linalyl acetate was performed by gas chromatography. Assay of total flavonoids of flamin was performed by spectrophotometric method with reference into isosalipurposide. Phytomedicine «Lavaflam» tablets meets European Pharmacopoeia requirements on the following parameters: appearance, geometrical sizes, average weight, disintegration, friability, resistance of tablets to crushing, quantification.

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