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Research Article

# COMPARATIVE STUDY OF THE ORIGINAL TECHNOLOGY OF MICRONIZATION OF THE PURIFIED FLAVONOID FRACTION OF "DETRALEX®" AND THE TECHNOLOGY OF MICRONIZATION OF DRUGS D AND N OF THE UKRAINIAN MANUFACTURERS

**IGOR ZUPANETS, SERGEY SHEBEKO, STANISLAV ZIMIN***

**Department of Clinical Pharmacology and Clinical Pharmacy, National University of Pharmacy, Kharkov, Ukraine.**
**Email: zimstasmich@gmail.com**

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**ABSTRACT**

**Objective:** The objective of the study was to compare the degree of micronization of the bioflavonoid fraction (90% diosmin and 10% hesperidin) of different manufacturers which used for the treatment of the chronic venous insufficiency.

**Methods:** "Detralex®," medicines N and D, 500 mg coated tablets each were used as studied objects. Microscopy of the tablets matrix of the investigational drugs was performed after spontaneous decomposition in a water solution at pH=6.8, using a modular light field microscope of the research class B-1000BF (Optika, Italy) with a digital camera Optikam HDMI Pro (Optika, Italy) with measuring of the size of the flavonoid fraction granules.

**Results :** About 92.8% of the granules of the "Detralex®" tablets matrix were presented by the smallest size of the granules (1–5 μ), unlike D and N, in which 12.9% and 10% of the same size granules were observed. Giant granule size (up to 50 μm) was discovered in D and N tablets matrix and no such granules size were found in the "Detralex®" tablets matrix.

**Conclusion:** Different degrees of micronization of the purified flavonoid fraction in the studied test samples indicate that the drugs D and N are not pharmaceutically equivalent to the original "Detralex®" drug and cannot be considered as copies without further research.

**Keywords:** Tablets matrix, Granules, Diosmin, Hesperidin, Chronic venous insufficiency.

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## INTRODUCTION

Chronic venous insufficiency (CVI) - a disease of the cardiovascular system manifested by venous congestion due to congenital or acquired defects of the venous wall (defects or functional insufficiency of venous valves, narrowing of the lumen, or its occlusion, etc.). CVI includes varicose veins, post-thrombotic syndrome, primary venous insufficiency, and compression syndromes. This disease characterized by a high prevalence of up to 42% among women and 18% among men [1]. Treatment should be complex and include compression techniques, conservative, and surgical methods. Bioflavonoids have a wide range of pharmacological activities [2,3] and among conservative methods of treatment of CVI, the use of diosmin and hesperidin caused by their powerful phlebotonic, anti-inflammatory, and other pharmacological properties. However, along with high pharmacological activity, diosmin has some pharmaceutical deficiencies, namely low solubility in water [4-6], which at 20°C is 0.0012 g/L [7]. Due to low solubility in water, diosmin is poor absorbed from the gastrointestinal tract. Consequently, to increase both bioavailability and efficiency, diosmin is increasingly micronized to obtain particles with a diameter of 2 μm [4,6,8].

Micronization is achieved with air jets operating at supersonic speeds to create repetitive collisions between particles, resulting in an average particle size of <2 μ. Micronization increases the rate of dissolution of diosmin and improves its metabolism, which, in turn, improves the metabolites responsible for its pharmacological activity [4,9].

The positive effect of micronization on the pharmacological activity of the micronized purified flavonoid fraction (MPFF), which consists of 90% diosmin and 10% hesperidin, has been demonstrated both in preclinical and clinical pharmacological studies [4,10]. In a clinical study [2,7], 500 mg of MPFF in dosing twice daily for 2 months improved clinical symptoms and reduced venous outflow rates compared with 300 mg of unmodified diosmin taken 3 times a day. Consequently, micronization is essential for the effective absorption of active compounds. That is why the purpose of our study was to compare the degree of micronization of the bioflavonoid fraction (90% diosmin and 10% hesperidin) of the original drug "Detralex®" and drugs of the various manufacturers of Ukraine.

## METHODS

This article does not contain any studies with human or animal subjects performed by any of the authors. The following investigational medicinal products containing bioflavonoids diosmin and hesperidin were used:

- "Detralex®," 500 mg coated tablets (15 tablets in a blister, 4 blister packs), Laboratories Servier Industry, France (p. 620747);
- Medicine N, coated tablets, 500 mg, No. 60 (10 tablets in a blister, 6 blisters in a pack), Ukraine (pp. FY160317);
- Medicine D, coated tablets, 500 mg; № 60 (10 tablets in a blister, 6 blister packs), Ukraine (pp. 162328).

Microscopy of the tablets matrix of the investigational drugs was performed after spontaneous decomposition in a water solution at pH=6.8, which corresponds to the human intestinal contents using a modular light field microscope of the research class B-1000BF (Optika, Italy) with a digital camera Optikam HDMI Pro (Optika, Italy). During the study with the help of special software, measurements of the flavonoid fraction granules were taken and distributed according to the size in percentage. Measurements made with magnification of ×400, ×600, and ×1000 in the field of view of the microscope on 10 micropreparations.

**RESULTS**

In the course of the study, it was found that the size of the granules of the bioflavonoid fraction in the investigated drugs was from 1 to 50 μ, while in the drug "Detralex®," most of the granules had a size of 1–5 μ (93% of the total number of granules), preparations D and N - 5–10 μ (43% and 69%, respectively) (Table 1).

The analysis of the micropreparations of the tablet matrix of drug "Detralex®" showed that it was formed by MPFF with a predominant size not exceeding 5 μ (Fig. 1). The diameter of most granules forming the background of micropreparations was within 2 μm (Figs. 2-5). In some micropreparations, granules of a flavonoid fraction which size was closer to 10 μ were found, but in rare cases (Figs. 1 and 2). At the same time, these elements were complex conglomerates of smaller particles of flavonoids. Unique elements that were >10 μm have not been observed in any of the micropreparations of the tablet matrix of "Detralex®" (Figs. 1-3 and 5). However, in some cases, complex granules of >10 μm were present, which cannot be considered as separate whole particles (Figs. 3 and 4).

A slightly different picture was observed in the micropreparations of the tablet matrix of the drug D. Besides, the small fraction of flavonoids up to 5 μ, a large number of solid granules in the range from 10 to 50 μ, as well as single huge granules of > 50 μm (Fig. 6), were observed in the most samples. In some micropreparations, the majority of the flavonoid fraction had sized up to 20 μ with a fraction of <5 μ insignificant at all (Fig. 7).

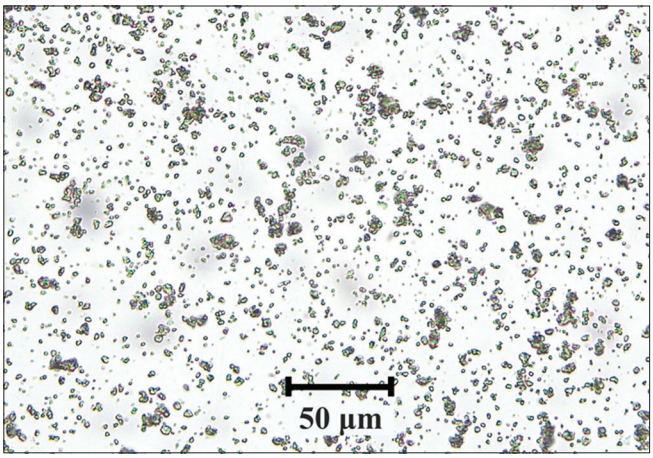


**Fig. 1: Micropreparation of the tablet matrix of the drug "Detralex®." A large number of granules with a size of not more than 5 μm. Single complex particles with sizes up to 10 μm. The absence of elements >10 μm ×400**

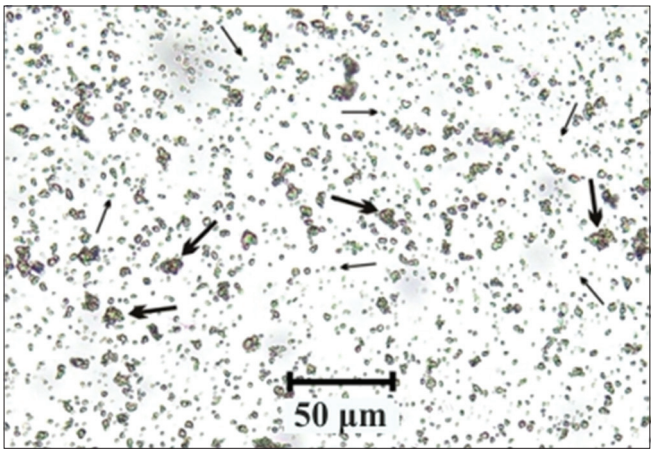


**Fig. 2: Micropreparation of the tablet matrix of the drug "Detralex.®" Flavonoid fraction granules with diameter 2 μm, forming a background for the all field of view (thin arrows). The presence of elements with diameter of about 10 μm from conglomerates of smaller particles (thick arrows) ×400**

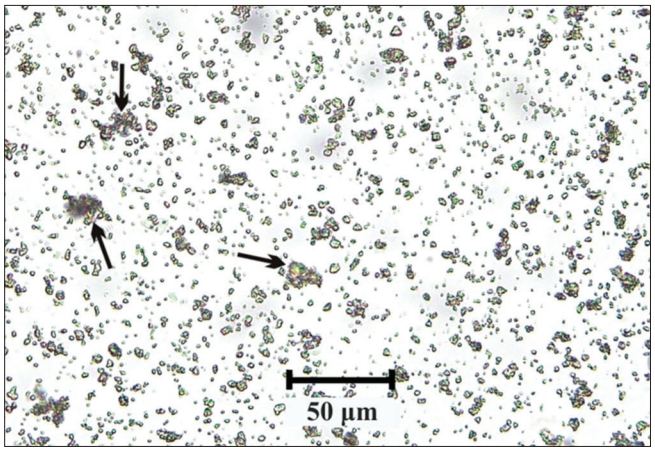


**Fig. 3: Micropreparation of the tablet matrix of the drug "Detralex®." Complex elements >10 μm (arrows). Granules with a size of 2 μm on all field of view ×400**

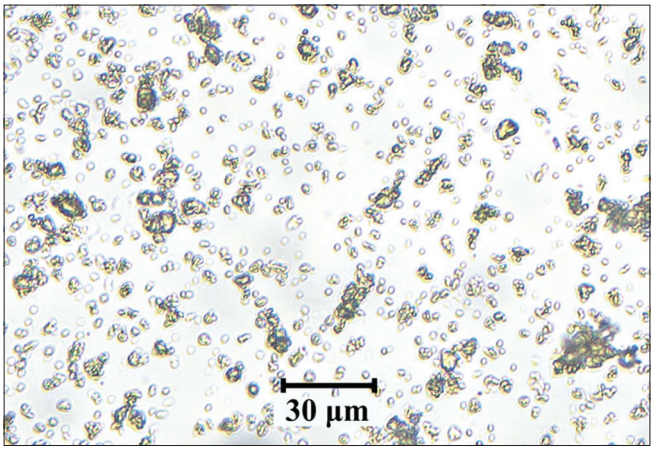


**Fig. 4: Micropreparation of the tablet matrix of the drug "Detralex®." A large number of granules of purified flavonoid fraction with diameter of 2 μm. Complex granules with diameter about 20 μm ×600**

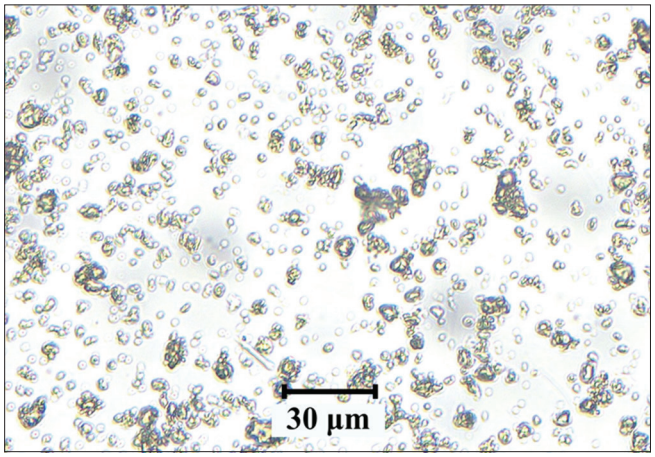


**Fig. 5: Micropreparation of the tablet matrix of the drug "Detralex®." Small particles of the flavonoid fraction. The absence of integral elements >10 μm ×600**

The described picture confirmed by the study of micropreparations of the tablet matrix of the drug D with a magnification of 600 times. There were a large number of flavonoids in the range of 10–20 μm. In addition, huge particles with a size of about 50 μm and even more found (Figs. 8 and 9). There was a small fraction with diameter of about 5 μ on the all field of view on some micropreparations (Fig. 8).

In the study of micropreparations of the tablet matrix of drug D in the maximum magnification - 1000 times, it was found that the smallest fraction of the tablet matrix consists predominantly of particles with dimensions of >2 μm, which is a fundamental difference from "Detralex®." In this case, most of the granules had a size of 5–10 μm (Figs. 10 and 11). When calculating the distribution of the particles of the tablet matrix, depending on the size, the following results were found: Fraction 1–5 μm was 12.9%, 5–10 μm - 42.5%, 10–20 μm - 27.7%, 20–50 μm - 15.7%, and >50 μm - 1.2% (Figs. 10 and 11, Table 1).

The obtained results are statistically credibly different from the results of studying of the tablet matrix of the "Detralex®" drug and indicate the different degrees and quality of the process of micronization in the manufacturing of these agents.

In the microscopic study of the tablet matrix of the drug N, there was a situation similar to the test samples of the drug D. A large fraction of flavonoids with a size of 5–10 μm with a minor fraction of 2 μm and a presence of giant granules with sizes > 50 μm observed in micropreparations at the magnification ×400 (Figs. 12 and 13).

All mentioned above differentiate structure of the MPFF of "Detralex®" and the drug N.

Practically, the complete absence of the smallest fraction of flavonoids - 2 μm or less also confirmed in the study of micropreparations of the tablet matrix of the drug N in the conditions of greater magnification - 600 times. The vast majority of particles

**Table 1: Distribution of the size of the granules of the purified flavonoid fraction of the tablet's matrix of "Detralex®," drugs D and N depending on size**

| The size of the granules, μm | Percentage | | |
|---|---|---|---|
| | "Detralex®" | D | N |
| 1–5 | 92.8±1.0 | 12.9±0.6* | 10.0±0.6* |
| 5–10 | 7.2±0.9 | 42.5±0.8* | 68.5±1.2* |
| 10–20 | 0 | 27.7±0.5* | 13.0±0.6* |
| 20–50 | 0 | 15.7±0.5* | 5.2±0.5* |
| >50 | 0 | 1.2±0.2* | 3.3±0.5* |

*Differences are reliably with the drug "Detralex®"

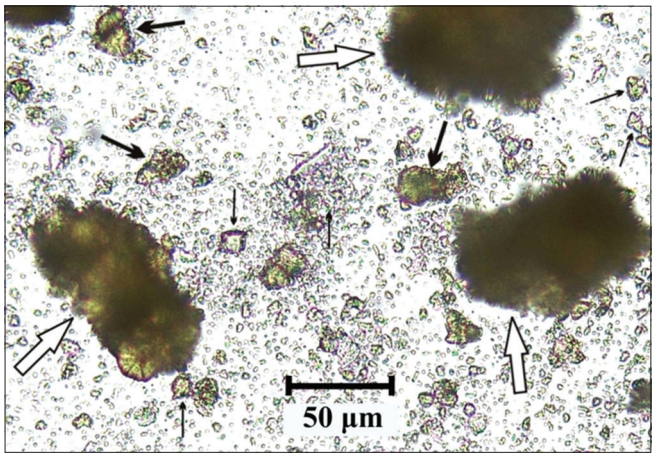


**Fig. 6: Micropreparation of the tablet matrix of the drug D. Small fraction of flavonoids up to 5 μm on all field of view. The presence of integral granules 10–20 μm (thin black arrows), 20–30 μm (thick black arrows), and >50 μm (white arrows) ×400**

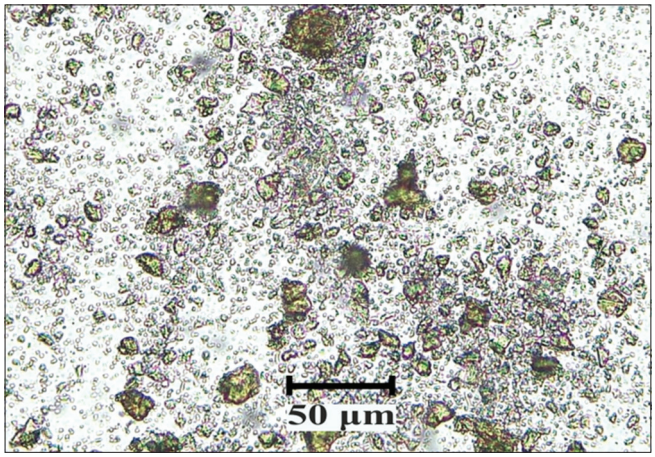


**Fig. 7: Micropreparation of the tablet matrix of the drug D. Most of the particles are in the range of 10–20 μm ×400**

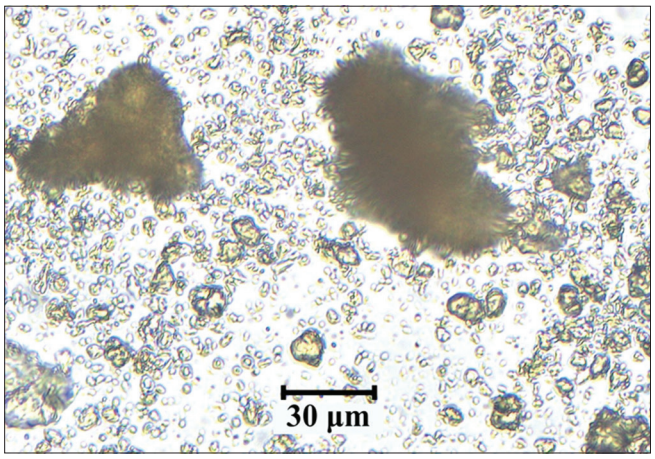


**Fig. 8: Micropreparation of the tablet matrix of the drug D. Small fraction up to 5 μm. A large number of granules sized 10–20 μm. Single granules over 30 μm ×600**

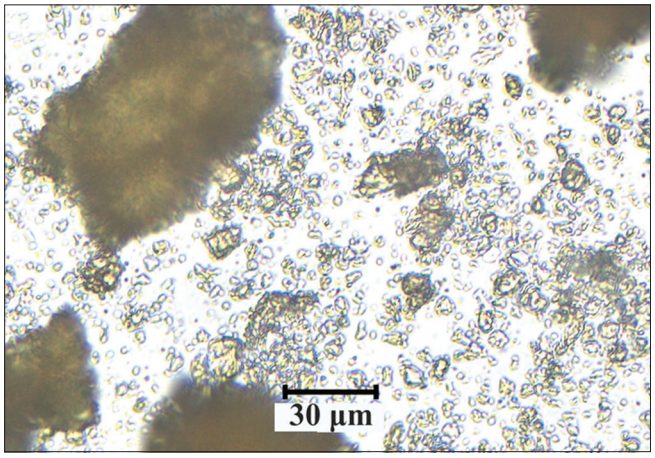


**Fig. 9: Micropreparation of the tablet matrix of the drug D. The majority of granules are 10–20 μm in size. The presence of giant granules with sizes over 50 μm ×600**

on these micropreparations had a size of 5–10 μm (Fig. 14). Granules larger 10–20 μm and 30 μm or more observed in some of them.

The results of the analysis of the distribution of the particles of the tablet matrix of the preparation N, depending on the size, indicate the following: The fraction 1–5 μm was 10.0%, 5–10 μm - 68.5%, 10–20 μm - 13.0%, 20–50 μm - 5, 2%, and >50 μm - 3, 3% (Figs. 15 and 16, Table 1).

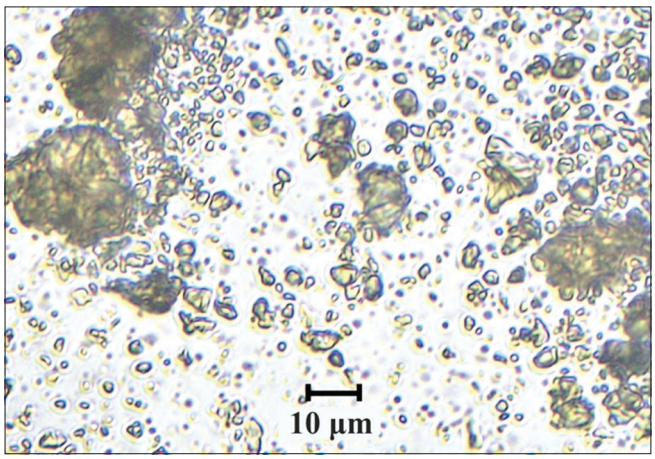


**Fig. 10: Micropreparation of the tablet matrix of the drug D. A cluster of particles with a size of 5–10 μm. Granules <2 μm are practically absent. The presence of integral granules with sizes >30 μm ×1000**

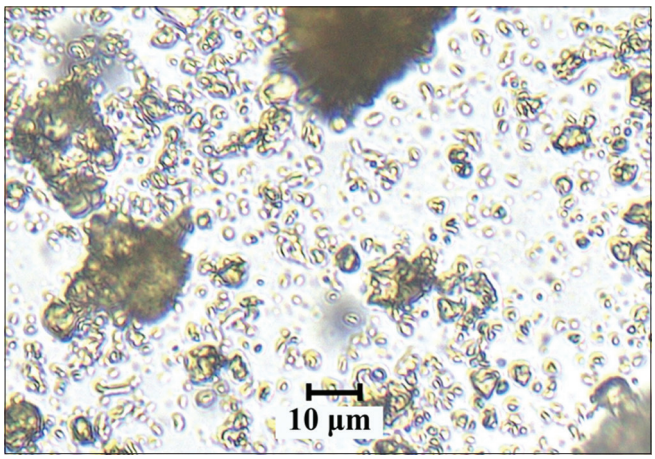


**Fig. 11: Micropreparation of the tablet matrix of the drug D. The vast majority of granules are 5–10 μm in size. A small number of particles sized <2 μm ×1000**

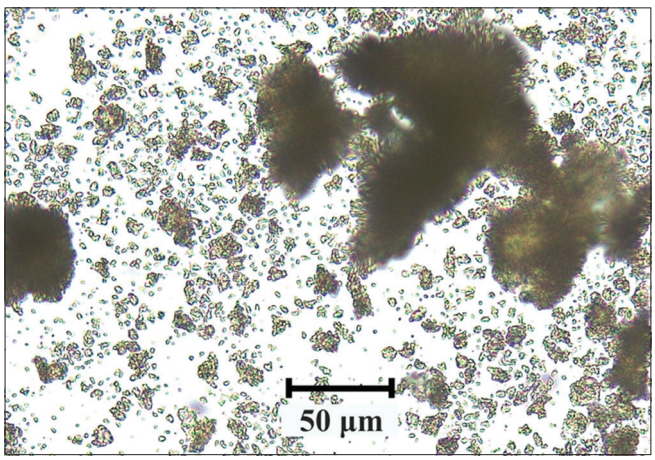


**Fig. 12: Micropreparation of the tablet matrix of the drug N. Particles with sizes 5–10 μm. Practically, the absence of granules less 2 μm. Giant coherent granules >50 μm ×400**

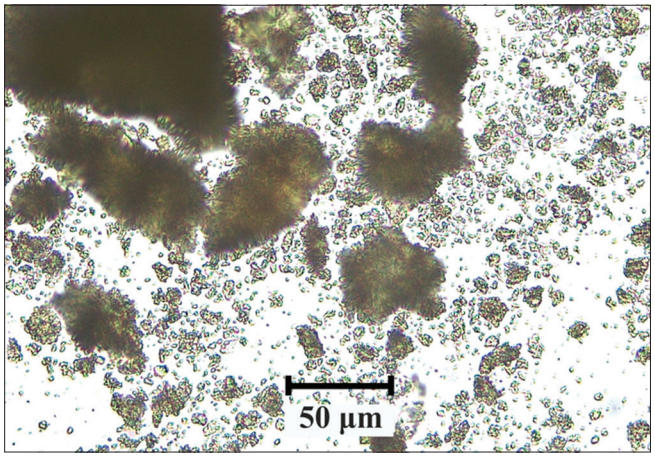


**Fig. 13: Micropreparation of the tablet matrix of the drug N. A large number of granules sized 50 μm or more. The small fraction is preferably 5–10 μm in size ×400**

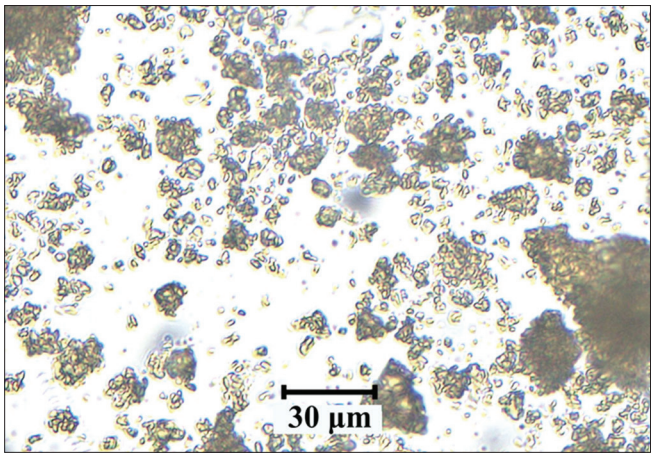


**Fig. 14: Micropreparation of the tablet matrix of the drug N. A large number of particles sized 5–10 μm. The presence of granules with sizes more than 30 μm ×600**

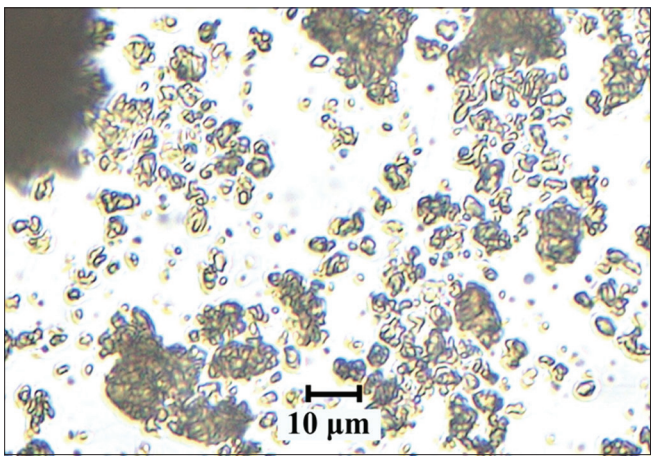


**Fig. 15: Micropreparation of the tablet matrix of the drug N. The vast majority of granules are 5–10 μm. Particles with diameter >10 μm ×1000**

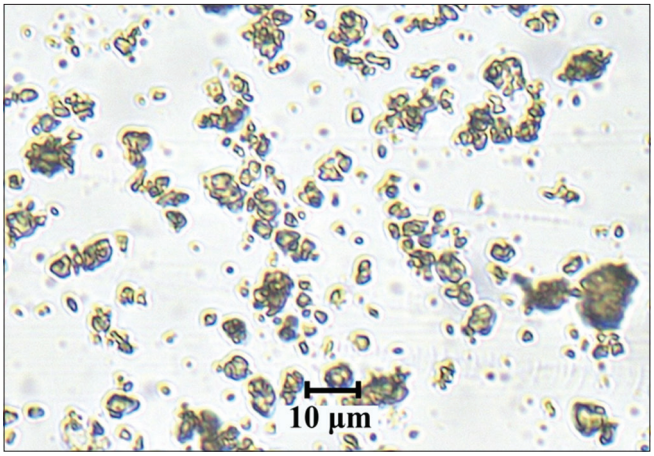


**Fig. 16: Micropreparation of the tablet matrix of the drug N. The vast majority of granules are 5–10 µm. A small amount of fraction of flavonoids <5 µm ×1000**

## DISCUSSION

The presented results testify to the statistical differences between the size of the particle of the tablets matrix of drugs D and N of Ukrainian manufacturing from the tablet matrix of the drug "Detralex®" manufactured by the Laboratory Servier Industrie, France, which evidence about the different quality of micronization process of these drugs.

It is known that one of the important parameters that affect the solubility and absorption of drugs is the surface area of the granule or the fraction of the tablet matrix after the dissolution of the tablet itself in the human's digestive tract. The area of the contact of drug and dissolvent depends on the square of granules surface, thus, the rate of dissolution of the drug [11]. However, not this square only, but the ratio volume(or mass)/surface square is important. The surface area of the granule with size 50 µ is larger than in a granule of 2 µ size in approximately 625 times (assuming that both granules are spherical). Hence, is it possible to assume that granules of 50 µ size are better and faster dissolve? No, it is impossible, because the ratio of the volume of particles (and hence, the mass at the same density) and the surface square of particles in the size of 2 µ is almost 24 times greater than that of particles with a size of 50 µ. Thus, reducing the size of the particles to be dissolved will increase this ratio and, in the end, the solubility and the rate of dissolution of the drug [12].

Particle size distribution, in turn, will also be more influence on the solubility, and therefore, on the rate and degree of absorption of the drug, which should lead to a more rapid and complete achievement of its maximum plasma concentration (Pmax). To make a reliable assessment of the effect of micronization on the main pharmacokinetic and pharmacodynamic parameters, appropriate *in vivo* comparative studies are necessary.

## CONCLUSIONS

Taking into account all of the above, we can draw the following conclusions:

- As a result of the microscopic study of the tablet matrix of the test samples of the drugs "Detralex®," D and N, a different degree of micronization of the purified flavonoid fraction identified. The content of particles of flavonoids sized 1–5 µ in "Detralex®" was 92.8%; in the medicine D - 12.9% and in the drug N - 10.0%; in the size of 5–10 µ in "Detralex®" - 7.2%; in the D medicine 42.5%; and in the preparation N - 68.5%; larger particles were not observed in the "Detralex®" test specimens, in contrast to which, particles in the test sample of other drugs were 10–20 µ (27.7% in the D medicine and 13.0% in the drug N), 20–50 µ (15.7% in the D and 5.2% in the N), and even >50 µ (1.2% and 3.3%, respectively);
- Microscopic analysis of the tablet matrix of the drug "Detralex®" showed the highest degree of micronization of the purified flavonoid fraction (the size of the particles of flavonoids was predominantly 2 µm), which indicates the unconditional benefits of this agent in the treatment of patients with varicose disease and hemorrhoids;
- Different degrees of micronization of the purified flavonoid fraction in the studied test samples indicate that the drugs D and N are not completely pharmaceutically equivalent to the original "Detralex®" drug and cannot be considered as copies without further research;
- The results of the conducted research indicate that the extrapolation of data on efficacy and safety obtained during preclinical and clinical study of "Detralex®," on medicines D and N is inadmissible.
- The appropriate *in vivo* comparative studies are necessary to assess the effect of degree and homogeneity of micronization on the pharmacokinetic and pharmacodynamics parameters.

## AUTHORS' CONTRIBUTIONS

Igor Zupanets - creating the idea of experiment, planning and supervising the experiment, and preparing an article. Sergey Shebeko - planning and executing the experiment, calculating data, and preparing an article. Stanislav Zimin - planning and executing the experiment, calculating data, and preparing an article.

## CONFLICTS OF INTEREST

The sponsor of scientific research is LLS "Servier." The sponsor of submitting of the manuscript is LLS "Servier."

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