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## СЪДЪРЖАНИЕ

### Оригинални статии

<i>A. M. Kottenko, A. I. Tikhonov, Yu. V. Chernykh, O. S. Shpichak и L. I. Tsebligina.</i> Използване на триизмерна флуоресцентна спектроскопия в проучването на лекарства на базата на медени продукти .....	3
<i>G. Momekov, P. Todorov, E. Naydenova, A. Kostovski и K. Troev.</i> Цитотоксична активност на нови $\alpha$ -аминофосфонови киселини при човешки малигнени клетъчни линии .....	9
<i>D. Terziivanov, K. Bozhinova, Em. Hristov, Iv. Atanasova и V. Lindareva.</i> Приложение на стратегията за D-оптималните времена и популационния фармакокинетичен анализ при биофармацевтично проучване на ампицилин .....	12
<i>V. Tzankova, B. Doncheva, S. Dragoni и M. Valoti.</i> Метаболизъм на 7-ethoxycoumarin в чернодробни срезове, получени по техниката "precision-cut" .....	17
<i>T. Dimitrov и N. Boyadjeva.</i> Сравнителна оценка на токсичността на Chlorpheniramine и Dexchlorpheniramine при мишки и плъхове и влиянието им върху хематологичните показатели при хроничната им токсичност при плъхове.....	23

### Обзори

<i>V. Slavova и N. Boyadjeva.</i> Полови хормони – фармакологични и терапевтични аспекти .....	31
<i>M. Draganova-Filipova, V. Saraftian и L. Peychev.</i> Влияние на прополис върху клетъчната пролиферация и имунния отговор.....	42

<b>Информационен отдел</b> .....	48
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## CONTENTS

### Original articles

<i>A. M. Kottenko, A. I. Tikhonov, Yu. V. Chernykh, O. S. Shpichak and L. I. Tsebligina.</i> Using three-dimensional fluorescent spectroscopy while studying drugs on the basis of apiculture products .....	3
<i>G. Momekov, P. Todorov, E. Naydenova, A. Kostovski and K. Troev.</i> Cytotoxic activity of new $\alpha$ -aminophosphonic acids against human malignant cell lines .....	9
<i>D. Terziivanov, K. Bozhinova, Em. Hristov, I. Atanasova and V. Lindareva.</i> Application of D-optimal timing strategy and population pharmacokinetic analysis in biopharmaceutical trials of ampicillin .....	12
<i>V. Tzankova, B. Doncheva, S. Dragoni and M. Valoti.</i> Metabolism of 7-ethoxycoumarin by rat precision-cut liver slices .....	17
<i>T. Dimitrov and N. Boyadjeva.</i> Comparative assessment of the toxicity of Chlorpheniramine and Dexchlorpheniramine in mice and rats and their influence on the hematological indices in the chronic toxicity in rats .....	23

### Reviews

<i>V. Slavova and N. Boyadjeva.</i> Sex hormones – pharmacological and therapeutic aspects .....	31
<i>M. Draganova-Filipova, V. Saraftian and L. Peychev.</i> Effects of propolis on cell proliferation and immune response.....	42

<b>Information section</b> .....	48
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## USING THREE-DIMENSIONAL FLUORESCENT SPECTROSCOPY WHILE STUDYING DRUGS ON THE BASIS OF APICULTURE PRODUCTS

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**Summary.** The three-dimensional fluorescence spectra of the drugs on the basis of apiculture products have been studied, the conformity of the spectra with the chemical composition of the drugs studied has been shown. Two intensive peaks of fluorescence in the range of  $\lambda_{em} - 320$  nm ( $\lambda_{exc} - 260$  nm) and  $\lambda_{em} - 345$  nm ( $\lambda_{exc} - 240$  nm) characteristic for the unsaturated fatty acids have been seen on 3-DF-spectrum of the bee pollen lipophilic extract. 3-DF-spectrum of the complex drug with the extract of the big bee moth biomass has the fluorescence peak in the range of  $\lambda_{em} - 315-330$  nm ( $\lambda_{exc} - 270$  nm), which is characteristic for the unsaturated lipids, as well as a number of peaks in the range of  $\lambda_{em} - 650-750$  nm ( $\lambda_{exc} - 400-430, 505, 540, 610, 650-680$  nm) characteristic for the mixture of chlorophylls A and B. While studying 3-DF-spectra of the individual components of suppositories with hydrophobic propolis drug and Hippophaë oil as well as their mixtures, it has been found that the points of the spectrum maxima of the suppositories correspond to the points of the spectrum maxima of the individual components; it testifies indirectly the absence of the chemical interaction between the components. The 3-DF-spectra of apidrugs obtained have the individual character and it gives the possibility of using them for identification.

**Key words:** three-dimensional fluorescent spectroscopy, bee pollen lipophilic extract, a complex drug with the extract of the larvae biomass of a big bee moth, suppositories with phenolic hydrophobic propolis drug (PHPD) and Hippophaë oil

## ИЗПОЛЗВАНЕ НА ТРИИЗМЕРНА ФЛУОРЕСЦЕНТНА СПЕКТРОСКОПИЯ В ПРОУЧВАНЕТО НА ЛЕКАРСТВА НА БАЗАТА НА МЕДЕНИ ПРОДУКТИ

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**Резюме.** Проучени са триизмерните флуоресцентни спектри на медикаменти, изготвени на базата на медени продукти. Показано е съответствието на спектрите с химичния състав на проучените лекарства. Два интензивни пика на флуоресценция в диапазона  $\lambda_{em} - 320$  nm ( $\lambda_{exc} - 260$  nm) и  $\lambda_{em} - 345$  nm ( $\lambda_{exc} - 240$  nm), характерни за ненаситените мастни киселини, са наблюдавани в 3-DF-спектъра на липофилния екстракт от пчелен полен. 3-DF-спектърът на комплексното лекарство с екстракта от биомасата на голямата *Galleria mellonella* имат пик на флуоресценция в диапазона  $\lambda_{em} - 315-330$  nm ( $\lambda_{exc} - 270$  nm), който е характерен за ненаситените липиди, както и няколко пика в диапазона  $\lambda_{em} - 650-750$  nm ( $\lambda_{exc} - 400-430, 505, 540, 610, 650-680$  nm), характерни за микстурата от хлорофил А и хлорофил Б. При проучването на 3-DF-спектрите на отделните съставки на супозитории с хидрофобно прополисово лекарство и масло от *Hippophaë*, както и на техните микстури, е установено, че точките от максималния спектър на супозиториите съответстват на точките от максималния спектър на отделните съставки. Това е непряко доказателство за липсата на химично взаимодействие между съставките. 3-DF-спектрите на получените на базата на меда лекарства имат индивидуални характеристики, което дава възможност за използването им за идентифициране.

**Ключови думи:** триизмерна флуоресцентна спектроскопия; липофилен екстракт от пчелен полен, комплексно лекарство с екстракт от биомаса на ларви на голямата *Galleria mellonella*, супозитории с фенолно хидрофобно прополисово лекарство (ФХПЛ) и масло от *Hippophaë*

## Introduction

Creating of medications on the basis of apiculture products is quite actual task at present due to their high biological activity, a wide spectrum of pharmacological action and their practically innocuous nature [1, 2, 5]. The multi-component structure and a certain dependence of the composition of apiculture products in the conditions of the vital activity of bees stipulate the necessity of a profound versatile study of the composition and an efficient quantitative determination of biologically active components with the purpose of apidrugs standardization.

For a number of decades, the Drug Technology Department of the National University of Pharmacy has been carrying out the program for developing medications with a direct therapeutical action on the basis of apiculture products under the supervision of academician A. I. Tikhonov. As a result of the long-term research, the methodological approaches of the standardized fractions isolation with the given pharmacological activity from the apiculture products have been developed; the formulation of different medicinal forms for apidrugs with the optimal bioavailability has been substantiated scientifically on the basis of studying physicochemical and technological properties of the substances. The complex wasteless technology of processing these precious products has been suggested [6].

The main biologically active components of propolis have been isolated as two standardized substances: phenolic hydrophobic propolis drug and phenolic hydrophilic propolis drug. A hydrophobic fraction contains flavones and flavonoles (luteolin, apigenin, quercetin, campferol, robidanol, etc.), it possesses a high antimicrobial activity, a marked anti-inflammatory, reparative and capillary – strengthening properties. A hydrophilic fraction comprises phenolcarboxylic acids, polysaccharides and oxycoumarins (coffeic, n-coumaric, erulic acids, scopoletin, esculetin, umbeliferon, etc.), it possesses a high antimicrobial and antiviral activity, has anti-inflammatory, analgesic and anti-oxidative action. Phenolic hydrophobic and hydrophilic fractions of propolis have become the main active components for a number of apidrugs: propolis tincture, Prolidoxide, Prolefen and Protrioxide ointments, Propolis, Propoltin and Propofen suppositories, Phenolen capsules, Propolin and Pheprogit tablets [7, 10].

The enzymatic and lipophilic fractions have been isolated from bee pollen by a complex processing. The main active component of the enzymatic

complex is  $\beta$ -fructofuranosidase. Polenzym tablets recommended for the substitutional therapy in a number of diseases have been developed on the basis of this complex. The bee pollen lipophilic extract (BPLE) contains the sum of carotinoids (200-320 mg % calculated by  $\beta$ -carotene), unsaturated fatty acids (10% calculated by the  $\alpha$ -linolic acid), tocoferols (300-400 mg % calculated by  $\alpha$ -tocoferol) and possesses a marked reparative, anti-inflammatory and membrano-strengthening action and antimicrobial activity. The curative and preventive creams, Lipovit ointment, Polenfen suppositories have been developed on the basis of the BPLE [4, 8].

A number of medications have been developed on the basis of bee venom, honey, mother milk: Melin, Lipophilized honey, Apufor, Apiven [6].

A comparatively new research direction of the department is development of medications on the basis of the larvae biomass of a big bee moth (wax moth), the product concomitant to apiculture. The pharmacological activity of the extract from bee moth larvae was found at the beginning of the last century by the outstanding Russian scientist I. I. Mechnikov [3]. A number of researchers proved the perspectiveness of their using as an immunomodulator in anemias of different genesis, toxicoses of the pregnant, infertility and tuberculosis; however, the absence of the standardized drug excluded the application of the product in medical practice [5]. On the basis of the research performed, the technology of the standardized extract of big bee moth biomass has been theoretically grounded and experimentally developed; its immuno-modulating and antimicrobial activities have been proven by the pharmacological research. The extract of big bee moth biomass has been included into the composition of the complex drug – Melofit, which also contains the extracts of *Glycyrrhiza glabra* roots, *Viola tricolor* and *Hypericum perforatum* herb. The main groups of the active substances of Melofit tincture are the complex of amino acids, fatty acids, ecdisterides, as well as chlorophylls A and B (3.2% calculated by chlorophyll A) and carotinoids (2.9% calculated by  $\beta$ -carotene). The pharmacological studies have proven that Melofit drug possesses immuno-modulating, antimicrobial and antituberculosis properties [11].

The multi-component structure of the composition of apiculture products causes the necessity of using them for their study and standardization of different methods of analysis; the presence of the compounds having their own fluorescence testifies the possibility of using the luminescence analysis for them.

In the analytical chemistry of the natural compounds, the modification methods are widely used. They are based on the determination of the maxima positions in the spectra of absorption, fluorescence and fluorescence excitation, the spectral characteristics of the representatives of the most widely spread groups of the natural compounds are well studied and given in the reference literature. However, the spectral identification methods are adapted either for individual compounds or for the mixtures of compounds belonging to one type and having the similar composition and the electron structure. The use of absorption spectra for the qualitative analysis of mixtures is not possible in the case of the mixtures isolated from the plant and animal material and containing the components with different spectral properties. In this case, the use of fluorescence spectra is more effective, though it is limited by the analysis of the fluorescent substances, it requires the selection of the lengths of excitation or fluorescence emission, and the spectra obtained may have the distorted form owing to the re-absorption.

The three-dimensional fluorescent spectroscopy (3-DF-spectroscopy) is a multi-factor method, which allows comparing the spectra of emission and fluorescence excitation, and thus, it can be used for the qualitative analysis of the fluorescent components in mixtures. Like the common fluorescence methods, 3-DF-spectroscopy has a high sensitivity (minimal limit of the components detection is up to  $10^{-7}$ - $10^{-6}$  mol/l depending on the quantum yield of the substance). However, unlike the other methods, 3-DF-spectroscopy allows identifying substances simultaneously by the position of the maxima of emission and fluorescence excitation (absorption) and it increases the reliability of identification.

The complete identification of all components of the plant and animal extracts is impossible, as a rule, and it is inexpedient in a number of cases. As the extract of each type has the unique chemical composition stipulated by the metabolism of the initial organism and the isolation conditions, it will have a distinctive unique 3-DF-spectrum. It allows identifying the extract under research in the composition of the more complicated mixtures without carrying out the complete determination of the extract's individual components.

*The aim of the paper* was the study of the possibility of using three-dimensional fluorescent spectroscopy for identification of the natural substances on the basis of apiculture products and control of the chemical interaction between the components in the complex multi-component drugs.

## Materials and methods

The objects of the research were the bee pollen lipophilic extract (BPLE), a complex Melofit drug, suppositories with the phenolic hydrophobic propolis drug (PHPD) and Hippophaë oil (the spectra of the components and their mixtures were determined separately for suppositories, as well as for the whole prescription developed).

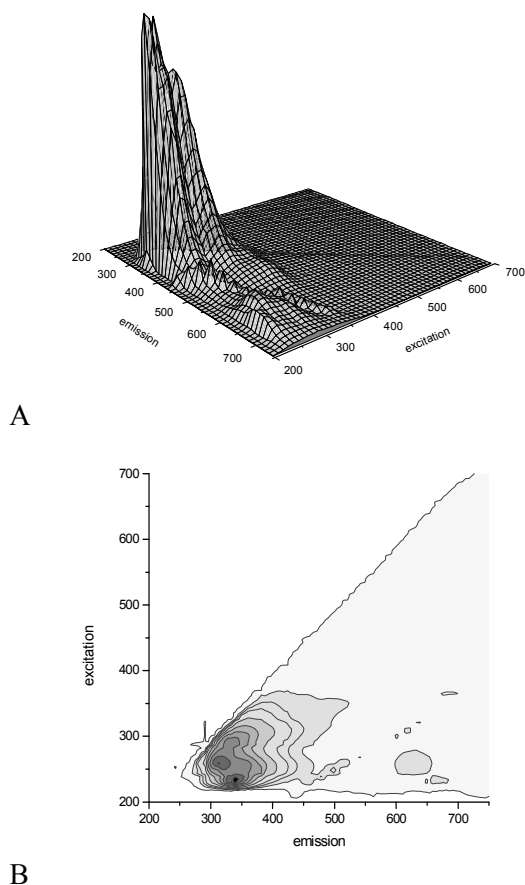
To prepare the standard solutions, 3-10 mg of the samples examined are dissolved in 5-10 ml of the solvent (chloroform or methanol) and diluted by the additional amount of the pure solvent to obtain the standard concentration with the optical density of 0.2-0.3 in the maxima of the absorption bands (the measurement was carried out by Hitachi U 3210 apparatus).

The 3-DF-spectra were taken in UV and visible range using Hitachi F 4010 spectrofluorometer re-programmed for performing 3-DF-measurements with the change of the exciting light length in the wave range from 220 (for solutions in methanol) or 250 (for solutions in chloroform) to 700-800 nm with the step of 5 nm. Further processing of the spectra was carried out using the Spectral Data Lab software developed by the Chemistry Scientific Research Institute at the Kharkov National University named after V. N. Karazin.

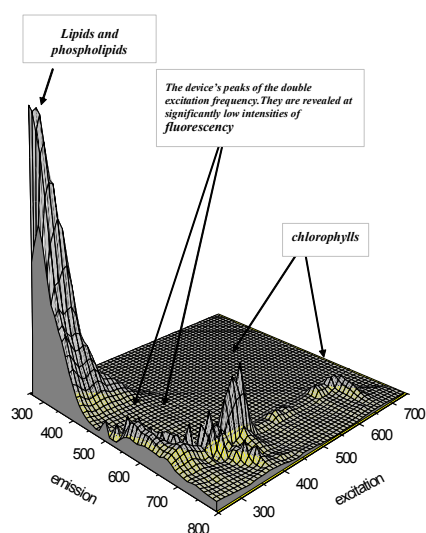
The computer processing allows subtracting 3-DF-spectrum of the solvent correctly from 3-DF-spectra of the standard solutions and obtaining 3-DF-spectrum of the sample as the values of the fluorescence intensity of the sample in the square matrix with the  $\lambda_{em}$ ,  $\lambda_{exc}$  axes. The data obtained can be presented either in the form of the three-dimensional picture of 3-DF-spectrum or its linear or logarithm projection on the  $\lambda_{em}$ ,  $\lambda_{exc}$  plane.

## Results and discussion

The three-dimensional fluorescence spectrum of BPLE and its projection are given in Fig. 1 (methanol as a solvent). Two intensive fluorescence peaks in the range of  $\lambda_{em} - 320$  nm ( $\lambda_{exc} - 260$  nm) and  $\lambda_{em} - 345$  nm ( $\lambda_{exc} - 240$  nm) are characteristic for the spectrum. According to the literature data, the first peak is characteristic for the unsaturated fatty acids and phospholipids and the second one is for the unsaturated fatty acids [9]. Taking into account the chemical composition of the BPLE determined earlier by other methods, in particular the presence of a great amount of the unsaturated fatty acids [4], we refer the given maxima in 3-DF-spectrum of the drug to the unsaturated fatty acids. Other fluorescent components of the drug are not revealed in the general spectrum of the drug because of their much less composition in the BPLE.



**Fig. 1.** Three-dimensional fluorescence spectrum of bee pollen lipophilic extract (A) and its projection (B)



**Fig. 2.** Three-dimensional fluorescence spectrum of a dry residue of Melofit complex drug.

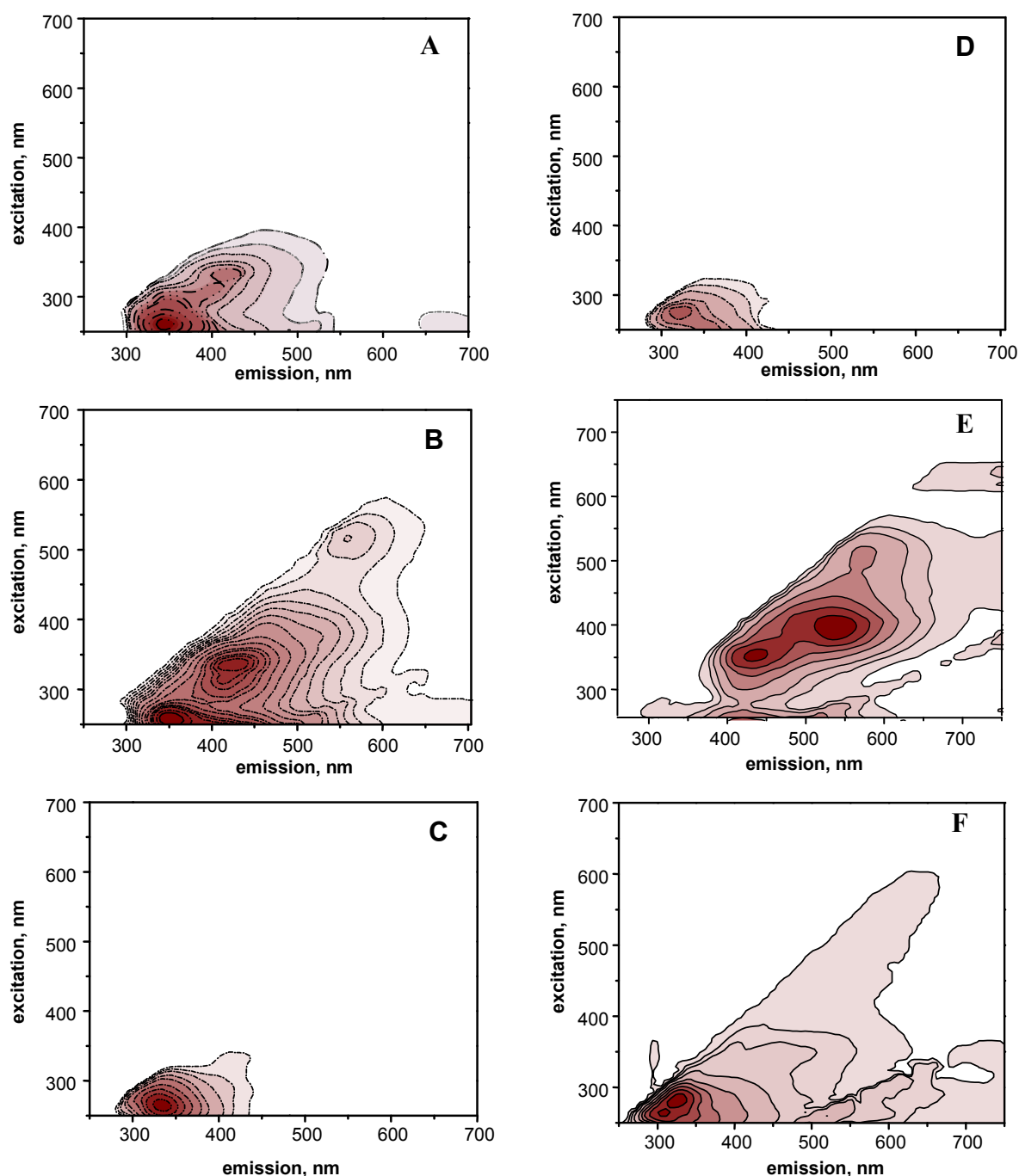
Several maxima are characteristic for the three-dimensional fluorescence spectrum of a dry residue of the Melofit complex tincture (chloroform as a

solvent). According to the literature data, the fluorescence peak in the range of  $\lambda_{em} - 315-330$  nm ( $\lambda_{exc} - 270$  nm) is characteristic for the spectra of simple phenolic compounds, unsaturated lipids and phospholipids, a number of peaks in the range of  $\lambda_{em} - 650-750$  nm ( $\lambda_{exc} - 400-430, 505, 540, 610, 650-680$  nm) corresponds to the mixture of chlorophylls A and B [9]. Taking into account the drug's composition found by other methods, the peaks mentioned in 3-DF-spectrum of the drug are referred to the unsaturated fatty acids and the mixture of chlorophylls A and B. The peaks revealing on 3-DF-spectrum in the range of  $\lambda_{em} - 480-600$  nm ( $\lambda_{exc} - 250-330$  nm) are instrumental ones of the double excitation frequency revealing at the low intensity of fluorescence.

The logarithm projection of 3-DF-spectra of suppositories with the PHPD and Hippophaë oil, as well as the individual components and their mixtures are given in Fig. 3 (methanol as a solvent). Taking into account that the spectrum of the physical mixture of compounds preserves the maxima points of the components, while these parameters usually change in the chemical interaction, we have carried out the comparison of the spectra of the individual components and their mixtures.

An insignificant fluorescence in the range of  $\lambda_{em} - 335-360$  nm ( $\lambda_{exc} - 250-270$  nm) is seen in the three-dimensional spectrum of the suppository base (the logarithm projection of 3-DF-spectrum is given in Fig. 3 D). As the base comprises more than 90% of the suppository mass, the given maximum is observed in all spectra of the mixtures (Fig. 3 A, B, C).

On the three-dimensional spectrum of the mixture of the suppository base and Hippophaë oil (Fig. 3 C), fluorescence of both components is added and becomes noticeably more intensive in the range of  $\lambda_{em} - 335-360$  nm ( $\lambda_{exc} - 250-270$  nm), the spectrum outline is also changed approximating to the outline of the pure Hippophaë oil spectrum (Fig. 3 F). The three-dimensional spectrum of the mixture of the suppository base with the PHPD (Fig. 3 B) has a characteristic fluorescence maximum for flavones and flavonols of the PHPD (Fig. 3 E) in the range of  $\lambda_{em} - 400-450$  nm ( $\lambda_{exc} - 325-360$  nm); the maximum characteristic to PHPD in the range of  $\lambda_{em} - 550-580$  nm ( $\lambda_{exc} - 500-530$  nm) is also remained.



**Fig. 3.** Logarithmic projection of the three-dimensional fluorescence spectrum of suppositories with PHPD and Hippophaë oil: **A** – suppository base with PHPD and Hippophaë oil; **B** – suppository base with PHPD; **C** – suppository base with Hippophaë oil; **D** – suppository base; **E** – PHPD; **F** – Hippophaë oil

The fluorescence peak of flavones and flavonols of the PHPD is well observed at the three-dimensional fluorescence spectrum of suppositories (suppository base with the PHPD and Hippophaë oil) in the range of  $\lambda_{em} - 400-450$  nm ( $\lambda_{exc} - 325-360$  nm); the weaker peak of PHPD is in the range of  $\lambda_{em} - 550-580$  nm ( $\lambda_{exc} - 500-530$  nm) and it masks with the stronger fluorescence of the components in other ranges of the spectrum. The points of the spectrum

maxima of the suppositories correspond to the points of the spectrum maxima of the individual components; it testifies indirectly the absence of the chemical interaction between the components.

Therefore, 3-DF-spectroscopy can be used when selecting the composition of the multi-component medications for the primary control of the absence of the chemical interaction between the individual components. The individual character of 3-DF-spectra

of the multi-component natural substances gives the possibility of using them as an additional identification method, especially while revealing adulterants.

### Conclusions

1. Three-dimensional fluorescence spectra of the BPLE, a complex drug Melofit and suppositories with the PHPD and Hippophaë oil have been studied. The conformity of the spectra obtained by the chemical composition of the drugs studied has been found; the individual character of 3-DF-spectra gives the possibility of using them as the identification method.

2. The conformity of the suppositories spectrum with the total sum of the individual components spectra has been shown by the consecutive study of 3-DF-spectra of the individual components and the complete composition of suppositories with the PHPD and Hippophaë oil. It proves the absence of interaction between the components and allows using the luminescence analysis for identification of the PHPD in the drug.

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