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# ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

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*RESEARCH ARTICLE*

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# IDENTIFICATION OF CANNABIMIMETIC MDMB(N)-073F METABOLITES IN URINE BY METHOD OF GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

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***Background.*** *At the beginning of 2019, the use of a new representative of synthetic cannabimimetics of the methylbutanoate indazole carboxamides group, MDMB(N)-073F, was recorded in a number of regions in the Russian Federation. Characteristic features of the pharmacological effect, the clinical picture of MDMB(N)-073F poisoning have not been studied, the psychoactive effects produced by MDMB(N)-073F remain unexplored. In this regard, the study of the new cannabimimetic metabolism is an important aspect in establishing the fact of taking MDMB(N)-073F during expert studies of biological objects.*

***The aim*** *of the research is identifying metabolites of synthetic MDMB(N)-73F cannabimimetics in real urine samples using solid-phase extraction (SPE) and gas chromatography (GC) with mass spectrometric detection (GC-MS).*

***Materials and methods.*** *10 urine samples were collected from March 15 to March 29, 2019. 8 urine samples were taken from the medical examination offices of the city of Yekaterinburg and the Sverdlovsk region from the persons examined for intoxication; 2 urine samples were obtained from the patients of the Sverdlovsk regional center of acute poisoning upon enrolment to the toxic-intensive care unit with a preliminary diagnosis of "acute poisoning by synthetic cannabimimetics". In the research, SampliQ EVIDEX-200 mg – 3 ml (Agilent, USA) cartridges were used for the sample preparation; β-glucuronidase Type HP-2, From Helix Pomatia, 100000 U/ml (Sigma-ALDRICH CHEMI, Germany) was used for enzymatic hydrolysis. Gas chromatography – mass spectrometry with the use of Agilent 7820 gas chromatograph with Agilent 5975 mass selective detector (Agilent, USA) was used as an instrumental method of the analysis.*

***Results.*** *The metabolites that make it possible to establish the fact of taking MDMB(N)-073F cannabimimetics via urine screening procedure to detect the presence of narcotic and medicinal substances with the use of solid-phase extraction and gas chromatography methods with mass spectrometry, have been described. The major metabolites MDMB(N)-073F in the urine of smoking mixtures consumers have been identified. The metabolism of MDMB(N)-073F has been found to be mainly due to hydrolysis of the ester group, hydroxylation, oxidative defluorination and N-dealkylation. Most of the resulting metabolites are excreted in the urine in the conjugated form.*

***Conclusion.*** *Gas chromatographic and mass spectrometric characteristics of some derivatives of the main metabolites of the new synthetic MDMB(N)-073F cannabimimetic have been obtained. This data can be used in the practice of forensic chemical and chemical toxicological analysis.*

***Keywords:*** *MDMB(N)-073F, cannabimimetics, metabolism, enzymatic hydrolysis, solid-phase extraction (SPE), gas chromatography – mass spectrometry*

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# ИДЕНТИФИКАЦИЯ МЕТАБОЛИТОВ КАННАБИМИМЕТИКА MDMB(N)-073F В МОЧЕ МЕТОДОМ ГАЗОВОЙ ХРОМАТОГРАФИИ С МАСС-СПЕКТРОМЕТРИЧЕСКИМ ДЕТЕКТИРОВАНИЕМ

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*В начале 2019 года в ряде областей Российской Федерации появился новый представитель синтетических каннабимиметиков группы метилбутаноатиндазолкарбоксамидов – MDMB(N)-073F. Особенности фармакологического действия, клиническая картина отравлений MDMB(N)-073F не изучены, психоактивные эффекты, производимые MDMB(N)-073F, являются неисследованными. В этой связи изучение метаболизма нового каннабимиметика является важным аспектом в установлении факта приема MDMB(N)-073F при экспертных исследованиях биологических объектов.*

***Цель исследования** – выявление метаболитов синтетического каннабимиметика MDMB(N)-073F в реальных образцах мочи с использованием твердофазной экстракции (ТФЭ) и газовой хроматографии с масс-спектрометрическим детектированием (ГХ-МС).*

***Материалы и методы.** 10 образцов мочи были собраны в период с 15 по 29 марта 2019г. 8 проб мочи были доставлены из кабинетов медицинского освидетельствования г. Екатеринбурга и Свердловской области от лиц, освидетельствуемых на состояние опьянения; 2 образца мочи были получены от пациентов Свердловского областного центра острых отравлений при поступлении в токсико-реанимационное отделение с предварительным диагнозом «острое отравление синтетическими каннабимиметиками». В исследовании для подготовки проб применялись патроны для ТФЭ SampliQ EVIDEX – 200 мг – 3 мл (Agilent, США), для ферментативного гидролиза использовалась β-глюкуронидаза, Type HP-2, From Helix Pomatia, 100000 ЕД/мл (Sigma-ALDRICH CHEMI, Германия), в качестве инструментального метода анализа – газовая хроматография – масс-спектрометрия с использованием газового хроматографа Agilent 7820 с масс-селективным детектором Agilent 5975 (Agilent, США).*

***Результаты и обсуждение.** Описаны метаболиты, позволяющие установить факт употребления каннабимиметика MDMB(N)-073F в процедуре скрининга мочи на наличие наркотических и лекарственных веществ с применением методов твердофазной экстракции и газовой хроматографии с масс-спектрометрией. Выполнена идентификация основных метаболитов MDMB(N)-073F в моче потребителей курительных смесей. Установлено, что метаболизм MDMB(N)-073F, главным образом, обусловлен гидролизом сложноэфирной группы, гидроксилированием, окислительным дефторированием и N-деалкилированием; большая часть образующихся метаболитов выводится с мочой в конъюгированном виде.*

***Заключение.** Получены газохроматографические и масс-спектрометрические характеристики некоторых производных основных метаболитов нового синтетического каннабимиметика MDMB(N)-073F, которые могут быть полезны в практике судебно-химического и химико-токсикологического анализа.*

***Ключевые слова:** MDMB(N)-073F, каннабимиметики, метаболизм, ферментативный гидролиз, твердофазная экстракция, газовая хроматография – масс-спектрометрия*

## INTRODUCTION

Legislative efforts in the field of control over the traffic of narcotic drugs and psychotropic substances, made over the last years, have reduced the scale of the emergence of the “designer drugs” series, but the trend towards the periodic emergence of new representatives of synthetic cannabimimetics remains. Synthetic cannabimimetics (SCs) are the most diversely represented

group of psychoactive substances on the illegal drug market. Over the past few years, several "generations" of synthetic cannabimimetics have changed.

Thus, according to the chemical structure, the majority of SCs identified in 2014–2015, were found in the following groups: naphthoylindoles, 1-amino-1-oxobutane indazole carboxamides and methyl butane-indindazole carboxamides [1].

At the beginning of 2019, a new representative of synthetic cannabimimetics of the methylbutanoate indazole carboxamides group, MDMB(N)-073F, which is a 4-fluorine derivative of the previously encountered MDMB(N)-073 [2], was recorded in a number of regions in the Russian Federation.

According to the Decree of the Government of the Russian Federation No. 1097 (dated October 12, 2015), a synthetic cannabimimetic MDMB(N)-073F is covered by List I of narcotic drugs, psychotropic substances and their precursors, which are controlled by the government [3]. Based on the chemical structure, MDMB(N)-073F is a derivative of 2-(1-butyl-1H-indazol-3-carboxamido) acetic acid.

The features of the pharmacological effect, the clinical picture of MDMB(N)-073F poisoning have not been studied, the psychoactive effects produced by MDMB(N)-073F remain unexplored. In this regard, the study of new cannabimimetic metabolism seems relevant in the practice of expert institutions engaged in chemical-toxicological and forensic-chemical analysis of the objects.

**THE AIM** of work is to identify metabolites of synthetic cannabimimetics MDMB(N)-073F in real urine samples using solid-phase extraction and gas chromatography with mass spectrometric detection.

## MATERIALS AND METHODS

### *Equipment*

- Gas chromatograph Agilent 7820 (capillary column HP-5MS, inner diameter 0.25 mm, length 30 m, thickness of film 0.25 µm) (Agilent, USA);
- mass-selective detector Agilent 5975 (Agilent, USA);
- 12 position SPE Vacuum Manifold System (Supelco)
- low vacuum pump (KNF lab LABOPORT, France);
- thermal block PE-4030 (Ekros, Russia);
- single-channel vaporizer PE-2300 ("Ecros", Russia);
- microshaker PE-2 ("Ecros", Russia);
- microwave Supra MWS-1824SW (Russia);
- solid -phase extraction cartridges SampliQ EVIDEX (200 mg-3 ml) (Agilent, USA);
- semi-automatic dispensers with 4–40, 40–200 µl and 0.2–1, 1–5 ml range.

### *Materials*

Bis-trimethylsilyl-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane; β-glucuronidase, Type HP-2, From Helix Pomatia, 100,000 U / ml (Sigma-ALDRICH CHEMI, Germany). The chemicals used in the study are of the "chemically pure" brand. The storage of the urine samples before the study was carried out at + 4°C.

### *Sample preparation*

10 urine samples were collected from March 15 to March 29, 2019. 8 urine samples were taken from the medical examination offices of the city of Yekaterinburg and the Sverdlovsk region from the persons examined for intoxication; 2 urine samples were obtained from the patients of the Sverdlovsk regional center of acute poisoning upon enrolment to the toxic-intensive care unit with a preliminary diagnosis of "acute poisoning by synthetic cannabimimetics".

The preparation of urine samples using enzymatic hydrolysis was carried out in the following way: 50 µl of each of alcohol solutions of inner standards (ethylmorphine hydrochloride (0,02 mg/ml), N-ethylbenzylamine (0,01 mg/ml) and hexenal (0,2 mg/ml) were added to 1 ml of urine samples. Next, for one parallel of urine samples, their preliminary preparation was carried out. Hereby, enzymatic hydrolysis was used. 250 µl of 1/15 M phosphate buffer (pH 6) and 25 µl of β-glucuronidase were added to the urine sample, then the vial was corked up and exposed to $45^0$C for 2 hours.

2 ml 1/15 M phosphate buffer (pH 4,8) was added to the urine samples without and after hydrolysis. The contents of the vials were centrifugated at 3000 rpm for 10 minutes, the centrifugate was separated from the residue.

For extraction, SPE cartriges SampliQ EVIDEX (200 mg/3 ml) with a mixed phase were used. Conditioning of a sorbent was conducted via succesive transfer of 2 ml of 95% ethanol and 2 ml of 1/15 M phosphate buffer (pH 4,8) through the cartridge. After that, the sample was downloaded at the speed of 1 ml/min.

Flushing was conducted in a successive manner: 1 ml of 1/15 M phosphate buffer (pH 4,8) and 1 ml of 10% ethanol. Drying the cartridge was carried out in vacuum for 20 minutes. Eluate I was derived via double transfer of 2 ml of n-hexane – ethylacetate (2:1) concoction through the cartridge. Eluate II was derived via double transfer of 2 ml of dichloromethane – 2-propanol – 25% ammonia (2:1:0.1) concoction. Eluates I and II were vaporized in a nitrogen flow at 45°C.

### *Derivatization and research*

*Methylation.* 500 µl of anhydrous acetone, 40 µl of iodomethane and 20– 25 mg of anhydrous potassium carbonate were added to the dry residue of eluate I, the vial was corked up and heated at 60°C for 60 minutes in the thermal block. The vial was then cooled down, the fluid fraction of the reactive concoction was separated and transferred into a clean vial, then vaporized in nitrogen flow at 40°C. The dry residue was dissolved in 100 µl of anhydrous ethylacetate and 1 µl of this solution was put into chromato-mass-spectrometer's injector.

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*Acetylation.* 40 µl of anhydrous pyridine and 60 µl of acetic anhydride (washing off the vial wallsides) were added to the dry residue of eluate II, the vial was corked up and exposed to miscowave emission in the oven at 560 watt for 5 minutes. After cooling down, the vial was opened and the surplus reagents were vaporized in nitrogen flow at ≤ 40°C. The dry residue was dissolved in 100 µl of anhydrous ethylacetate, and 1 µl of this solution was put into the chromato-mass-spectrometer's injector.

*Trimethylsilyl esters acquisition.* 100 µl of BSTFA containing 1% of trimethylchlorsilane was added to the dry residue of eluate I or II, the vial was corked up, shaken with the microshaker and heated at 80°C for 60 minutes in the thermal block. The vial was cooled down and 2 µl was put into the chromato-mass-spectrometer's injector.

### *Operation mode of gas chromatograph with a mass-selective detector*

The speed of the flow of the carrier gas (helium) passing the column was 1.5 ml/min, the flow-splitting was 15:1 with the impulse delay of 1 minute after the sample injection. The temperatures of the injection port and the line connecting to the mass spectrometer were 250°C and 280°C, respectively. The initial temperature of the column was 70°C for 2 minutes; then, the column was heated up to 280°C at the rate of 20 degrees/min and kept at the final temperature for 8 minutes.

The voltage of the multiplier of the mass-spectrometric detector was equal to that of the automatic routine adjustment of the detector. The registration of mass spectrum for acetyl and methyl derivatives in full ion scanning mode was in mass range of 42–450 a.u. The registration of mass spectrum for trimethylsilyl derivatives in full ion scanning mode was in the mass range of 43–650 a.u.

Processing of the chromatograms in order to identify the components of the samples was carried out using *MSD ChemStation E.02.01.1177* (*Agilent*) и *AMDIS* (The Automatic Mass Spectral Deconvolution and Identification System, NIST) software.

The degree of conjugation of MDMB(N)-073F metabolites was determined for their methyl esters by the ratio of the peak area of the ion with the following m/z values: for M1 and M4 artifact – 219, for M2 – 249, for M3 – 159, for M5 – 245, for M6 – 235, for M7 and for M9 – 189, M8 – 217 ion. For N-methylhexenal (inner standard) in eluate I of urine with and without hydrolysis the ion peak area was 235.

The relative content for their trimethylsilyl esters M1, M10, M4 and artifact M4 was determined by internal normalization with respect to the peak area of the ion with the value of m/z 219 in urine eluate I with enzymatic hydrolysis.

The results of the calculations of physicochemical constants (LogP, $K_{OC}$) were obtained using the software package *ACD/Labs v6.0* (*Advanced Chemistry Development Inc.*, Toronto, Canada).

## RESULTS AND DISCUSSION

The chemical name of cannabimimetics is MDMB(N)-073F – 2-[1-(4-fluorobutyl)-1H-indazole-3-carboxamide]-3,3-dimethylbutanoic acid methyl ester; the chemical formula is: $C_{19}H_{26}FN_3O_3$; the molecular mass = 363,4 g/mol. Its synonyms are: 4-fluoro MDMB-BINACA, 4F-MDMB-BINACA, 4-fluoro MDMB-BUTINACA.

MDMB(N)-073F is a derivative of the already known compound MDMB(N)-073 [2] and differs from the latter by the presence of fluorine in position 4 of the alkyl substituent of the indazole heterocycle. The chemical structures of cannabimimetics MDMB(N)-073 and MDMB(N)-073F are shown in Fig. 1.

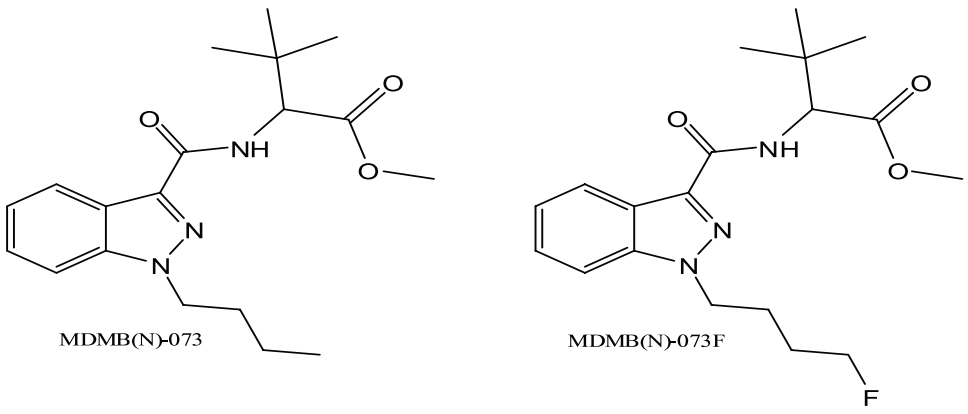


***Figure 1 – Chemical structures of MDMB(N)-073 and MDMB(N)-073F cannabimimetics***

The supposed chemical structure of MDMB(N)-073F metabolites, identified during the examinations of urine samples of the individuals who used smoking mixtures, is shown in Fig. 2.

The structures of metabolites were determined on the basis of mass fragmentation of peaks, which were detected on chromatograms obtained in the study of urine samples of drug users. The structures were also determined

on the basis of literature data on mass fragmentation of metabolites MDMB(N)-073 [2] and 5F-AB-PINACA [4]. In order to establish the properties of functional groups in the structure of metabolites, various types of derivatization were used, as well as their sequential combination.

Fig. 3–16 present the supposed structures and mass-spectra of M1-M10 MDMB(N)-073F metabolite derivatives.

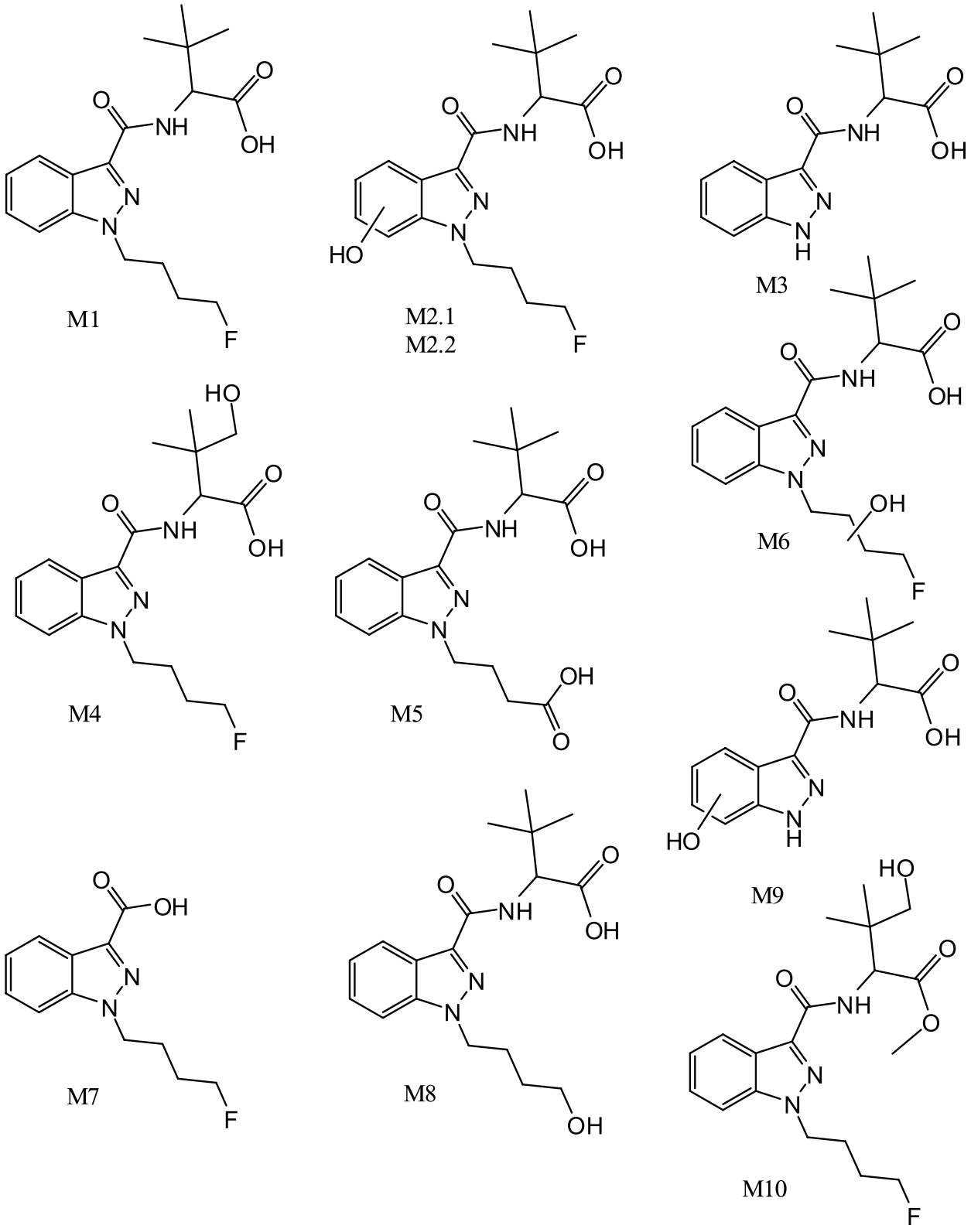


***Figure 2 – Supposed chemical structures of cannabimimetic MDMB(N)-073F metabolites***

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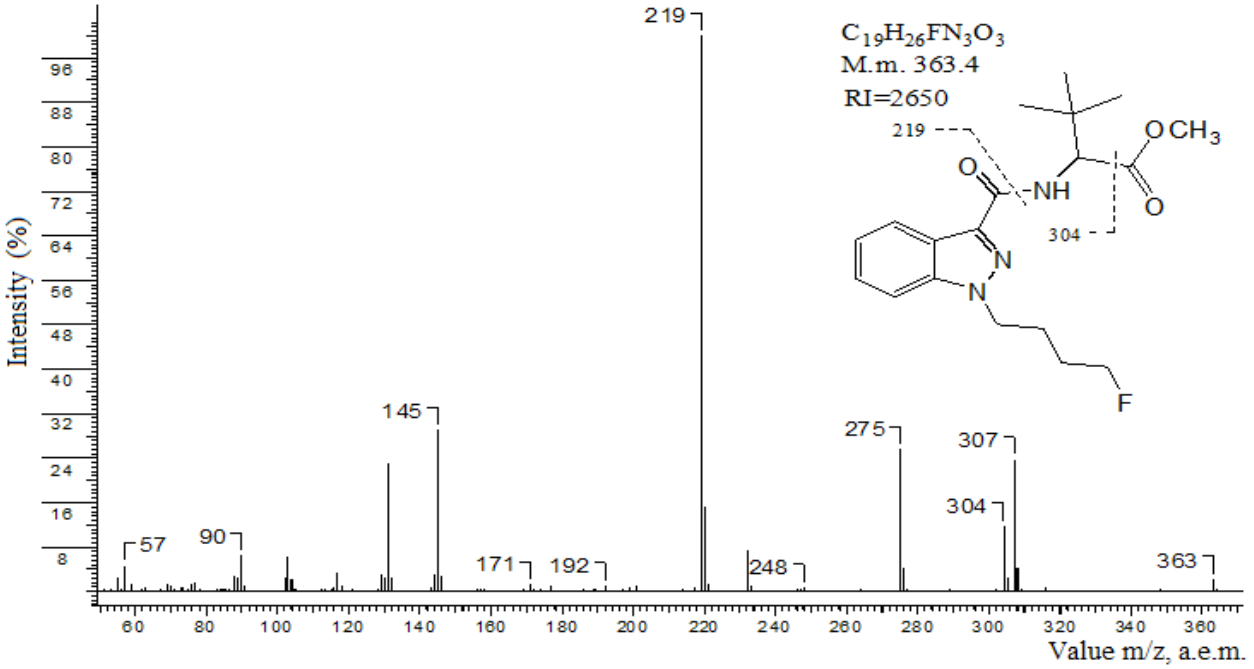


*Figure 3 – Mass-spectrum, retention index and structure of methyl ester of M1 metabolite*

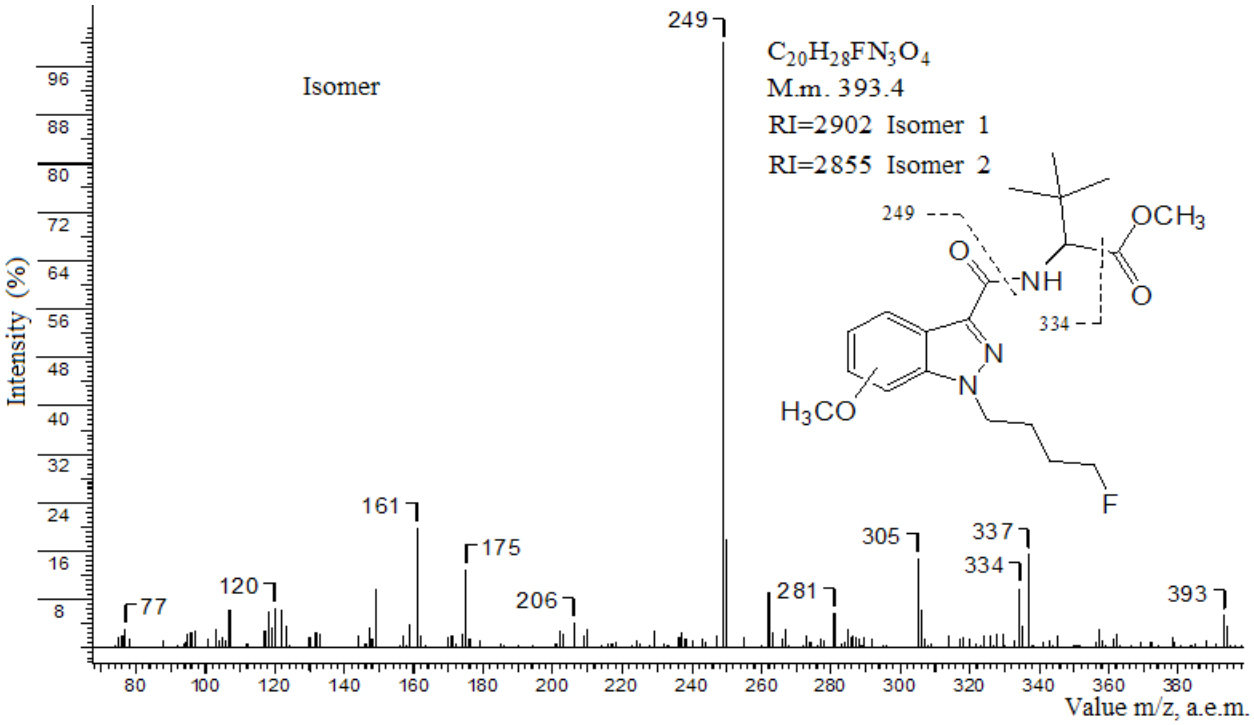


*Figure 4 – Mass-spectrum, retention index and structure of methyl ether of M2.2. metabolite*

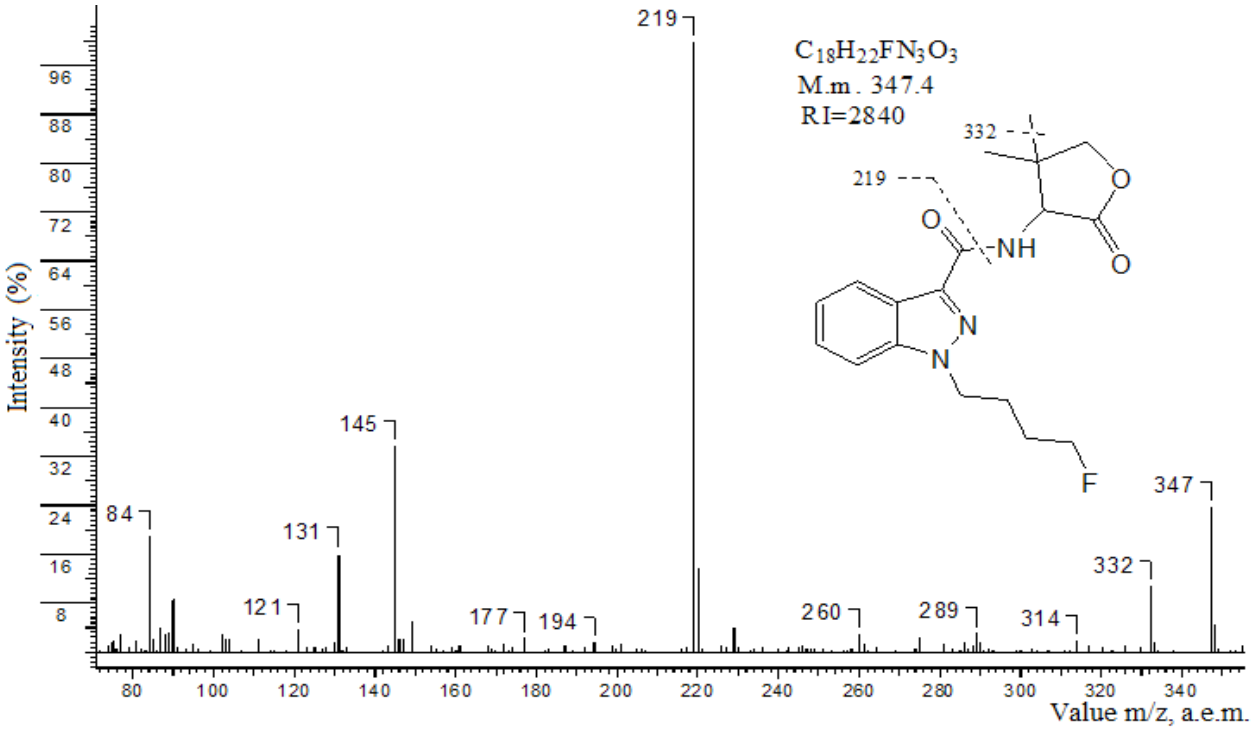


*Figure 5 – Mass-spectrum, retention index and structure of dimethyl derivative M3*

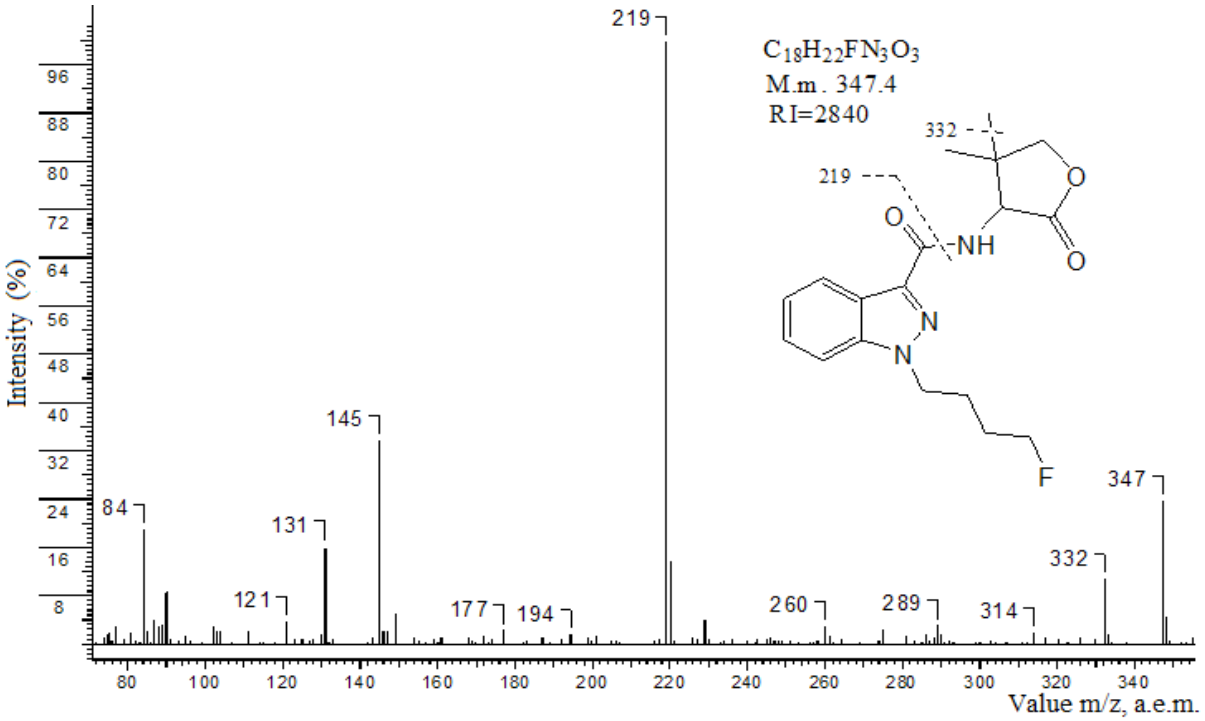


*Figure 6 – Mass-spectrum, retention index and structure of M4 metabolite artifact*

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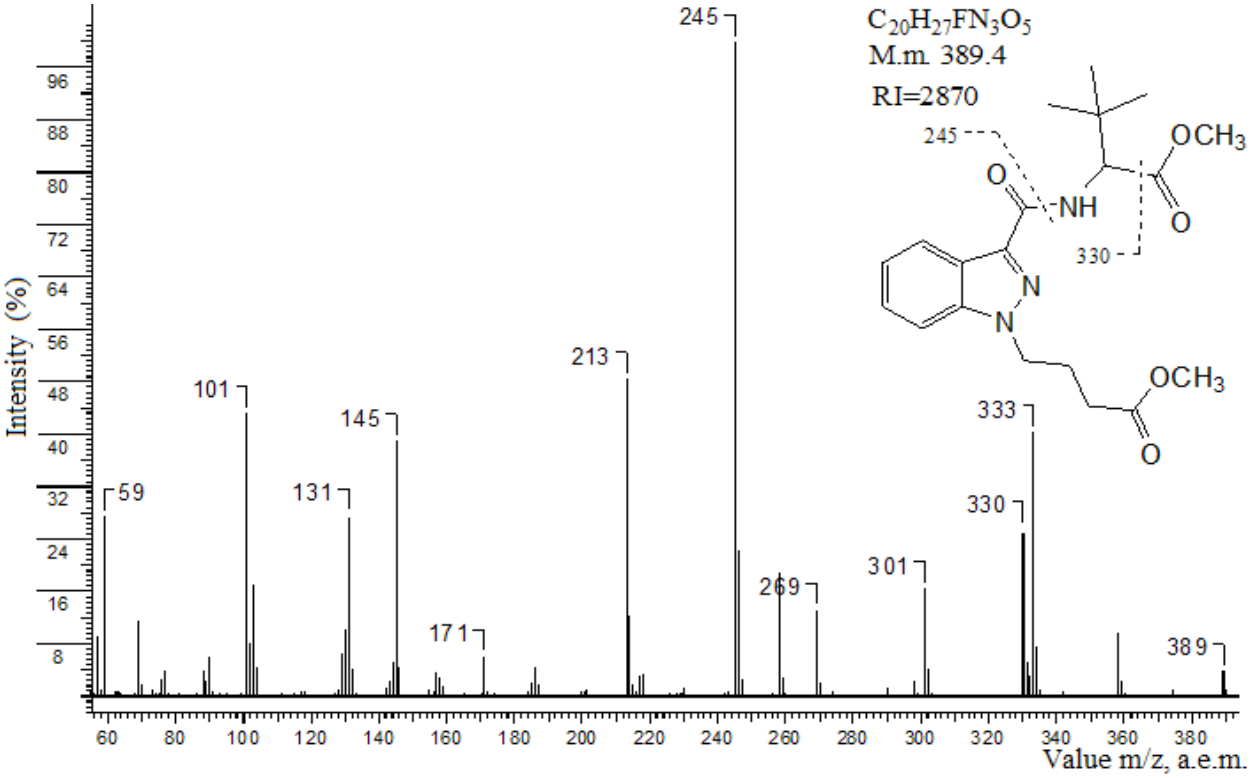


*Figure 7 – Mass-spectrum, retention index and structure of dimethyl ester of M5 metabolite*

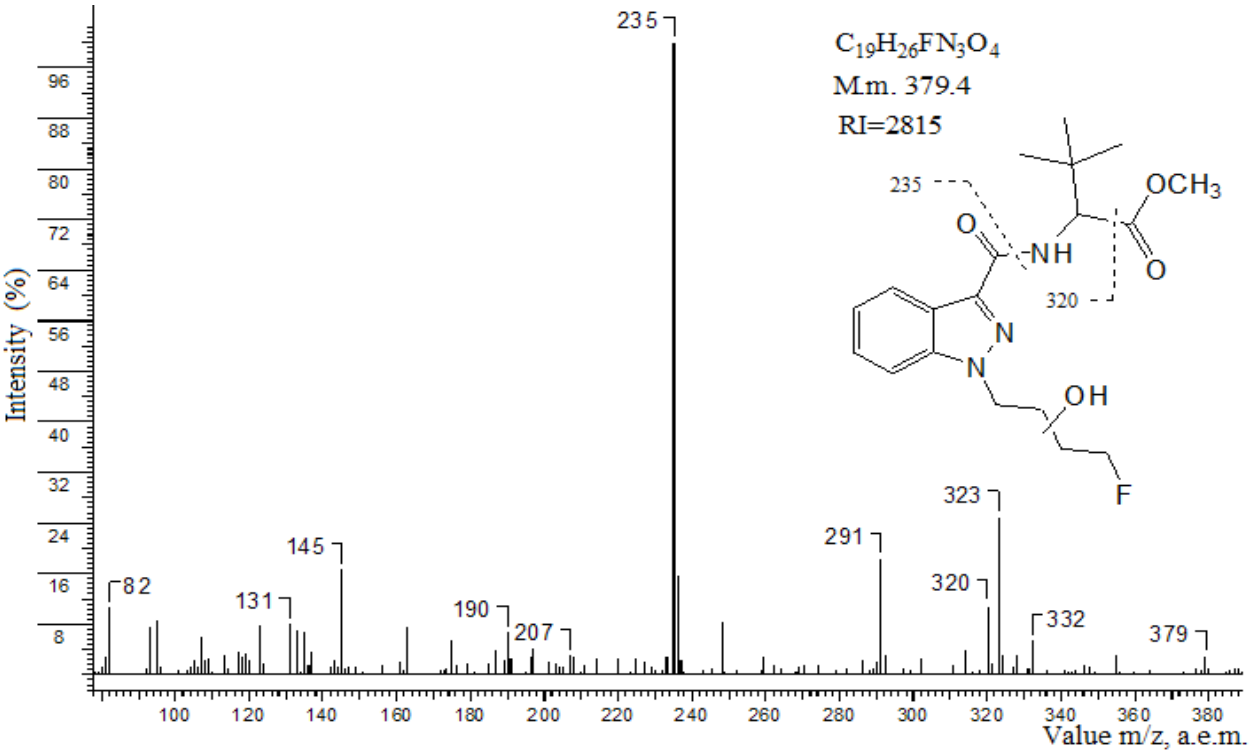


*Figure 8 – Mass-spectrum, retention index and structure of methyl ester of M6 metabolite*

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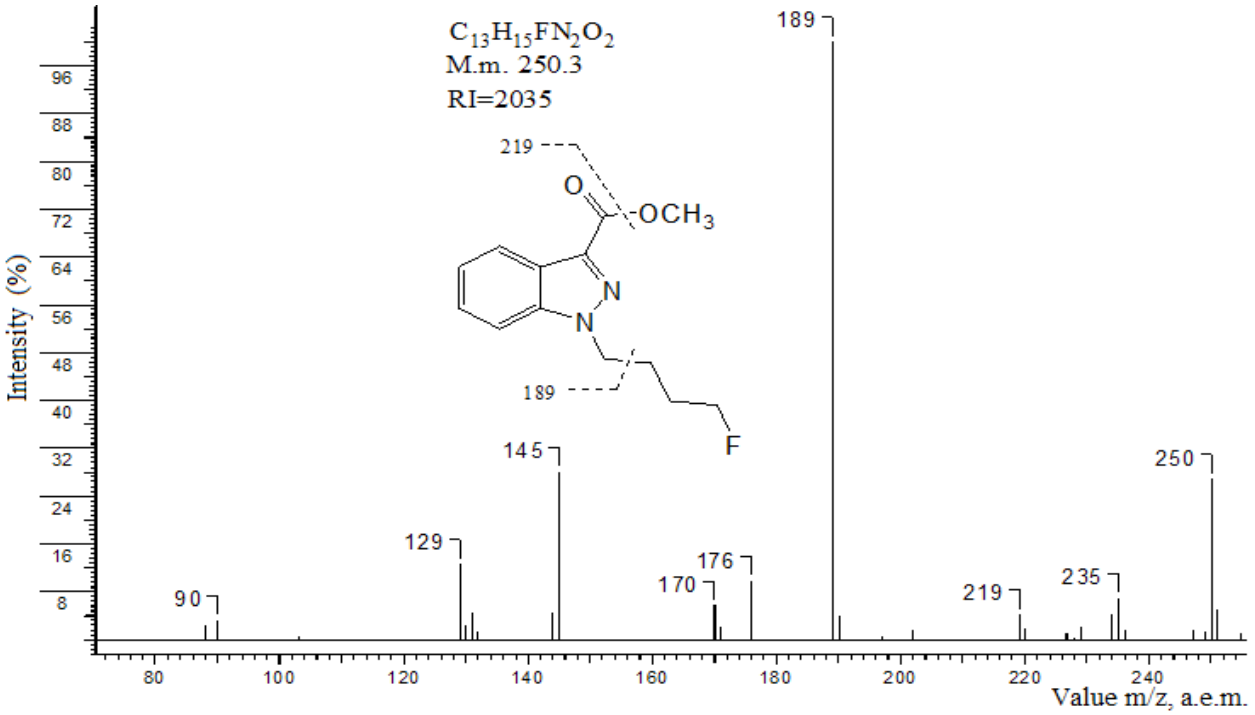


***Figure 9 – Mass-spectrum, retention index and structure of methyl ether of M7 metabolite***

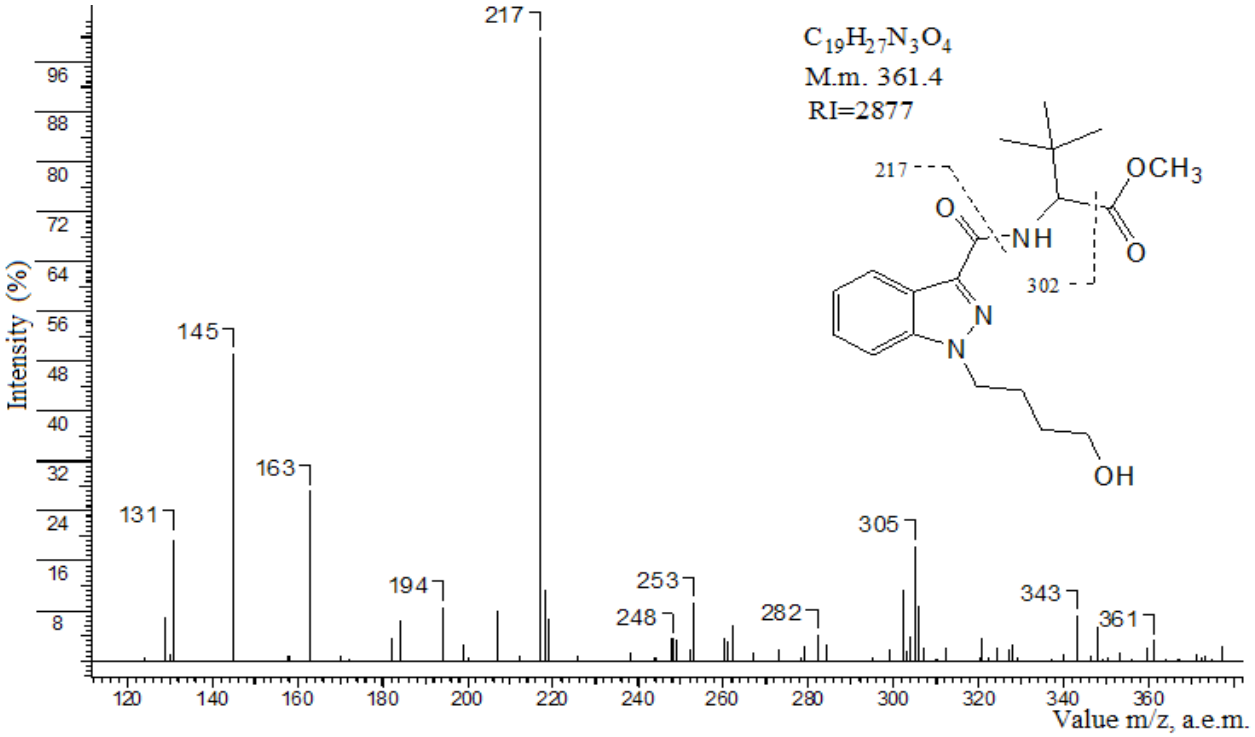


***Figure 10 – Mass-spectrum, retention index and structure of methyl ether of M8 metabolite***

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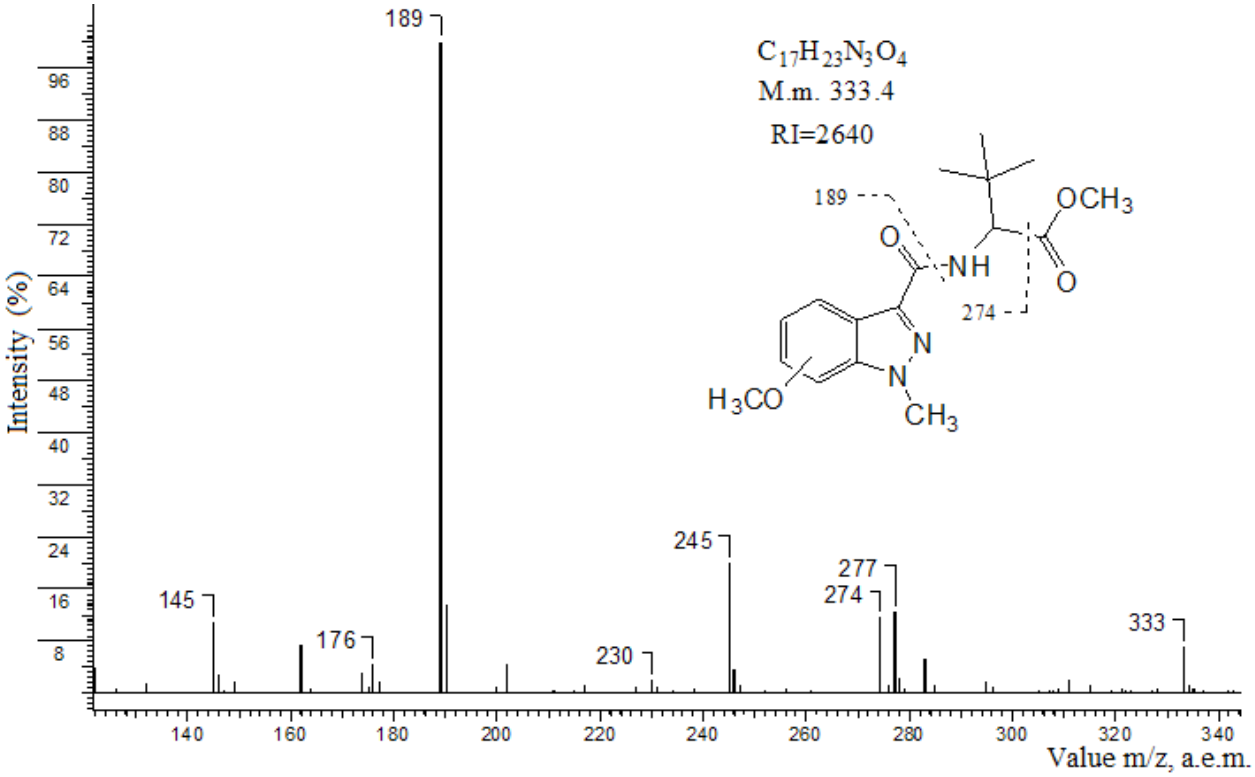


*Figure 11 – Mass-spectrum, retention index and structure of trimethyl derivative of M9 metabolite*

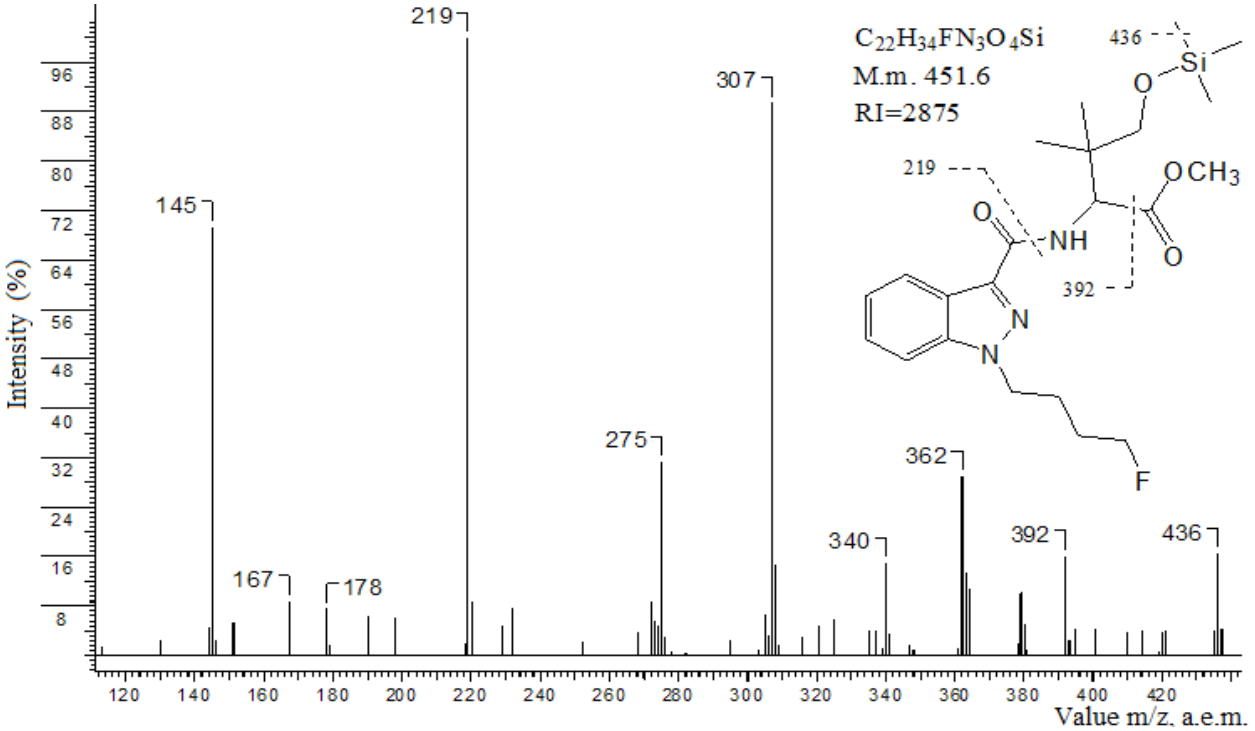


*Figure 12 – Mass-spectrum, retention index and structure of trimethylsilyl ester of M10 metabolite*

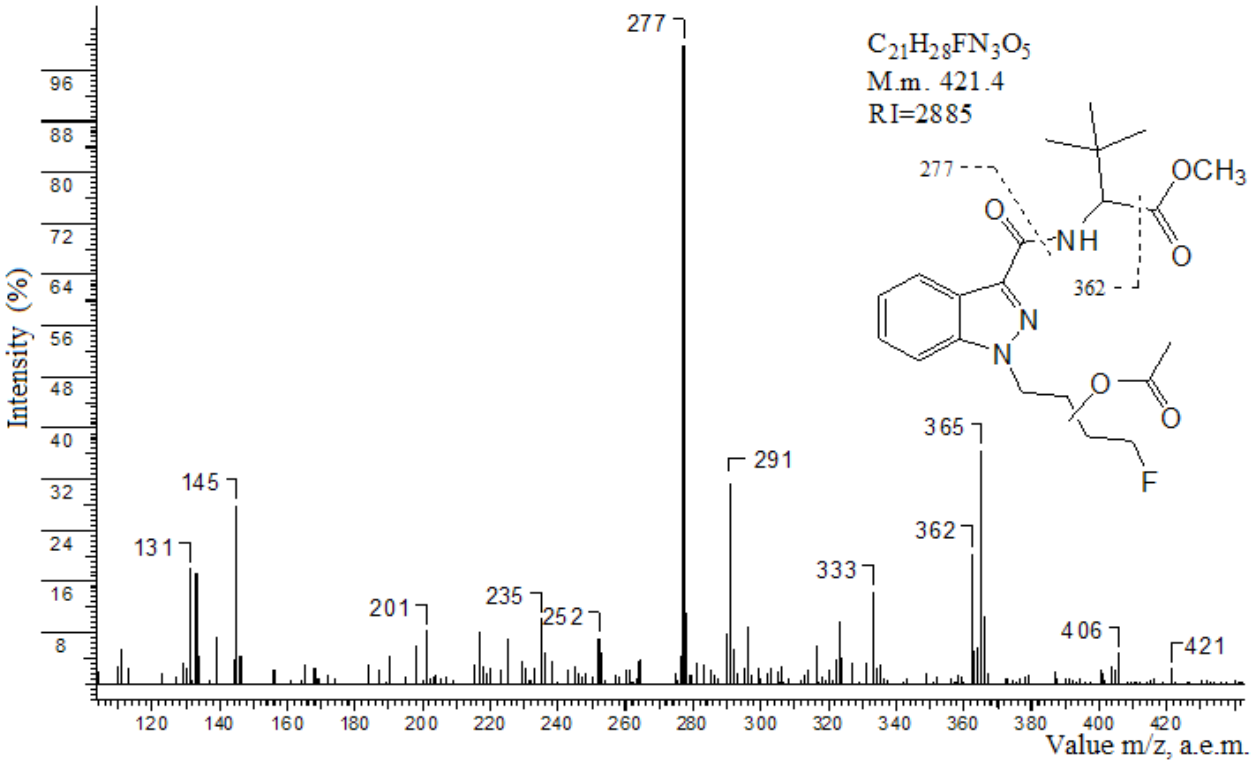


*Figure 13 – Mass-spectrum, retention index and structure of monomethyl ether of M6 metabolite after acetylation*

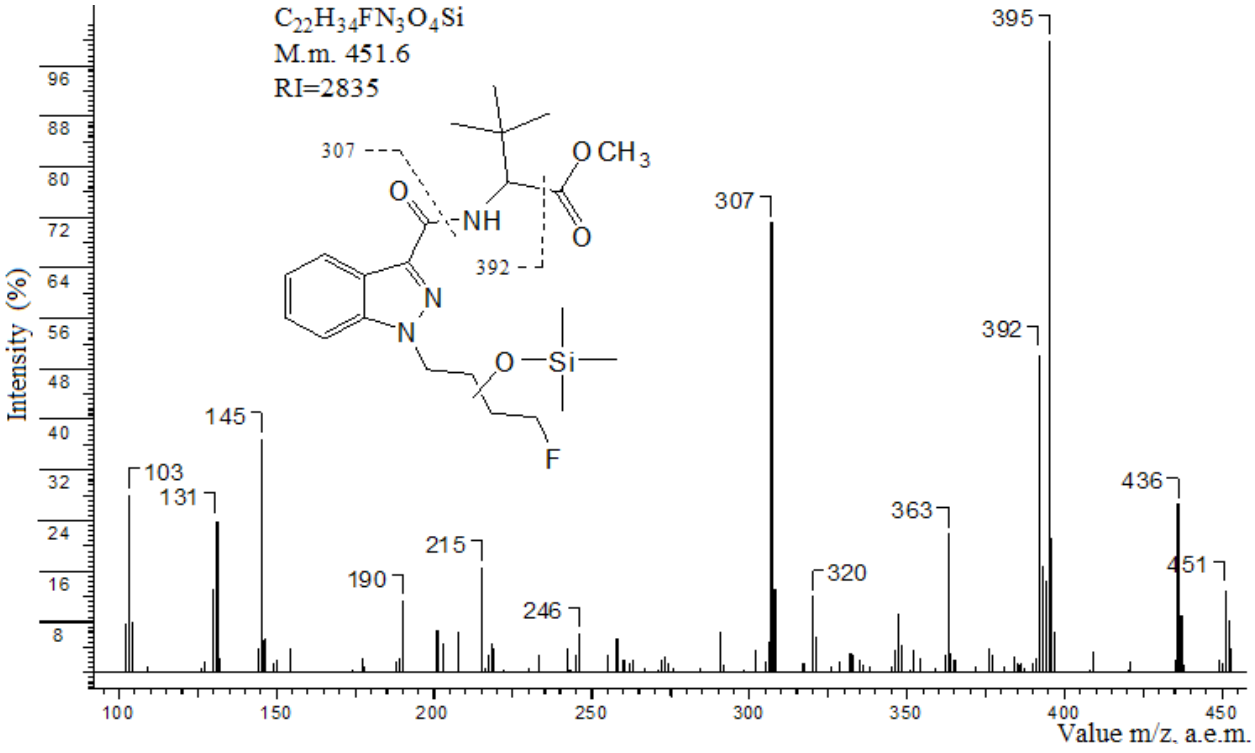


*Figure 14 – Mass-spectrum, retention index and structure of monomethyl ether of M6 metabolite after treating with BSTFA*

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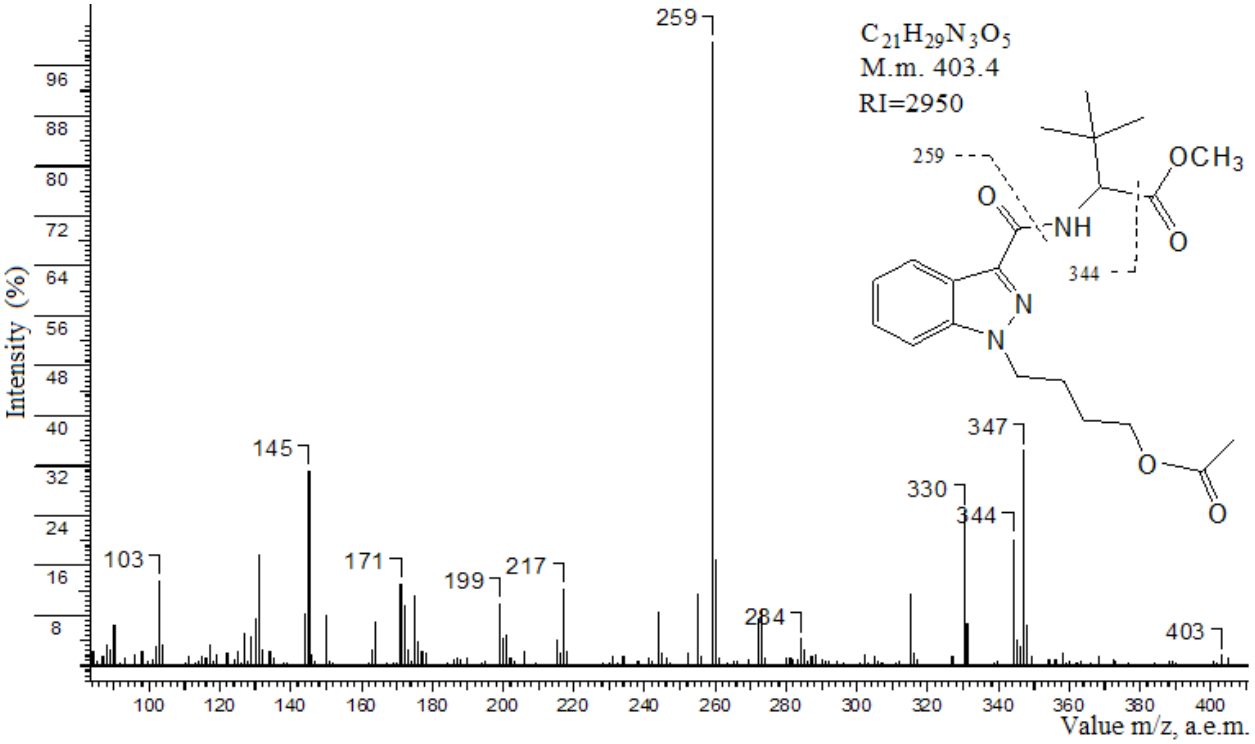


***Figure 15 – Mass-spectrum, retention index and structure of monomethyl ester of M8 metabolite after acetylation***

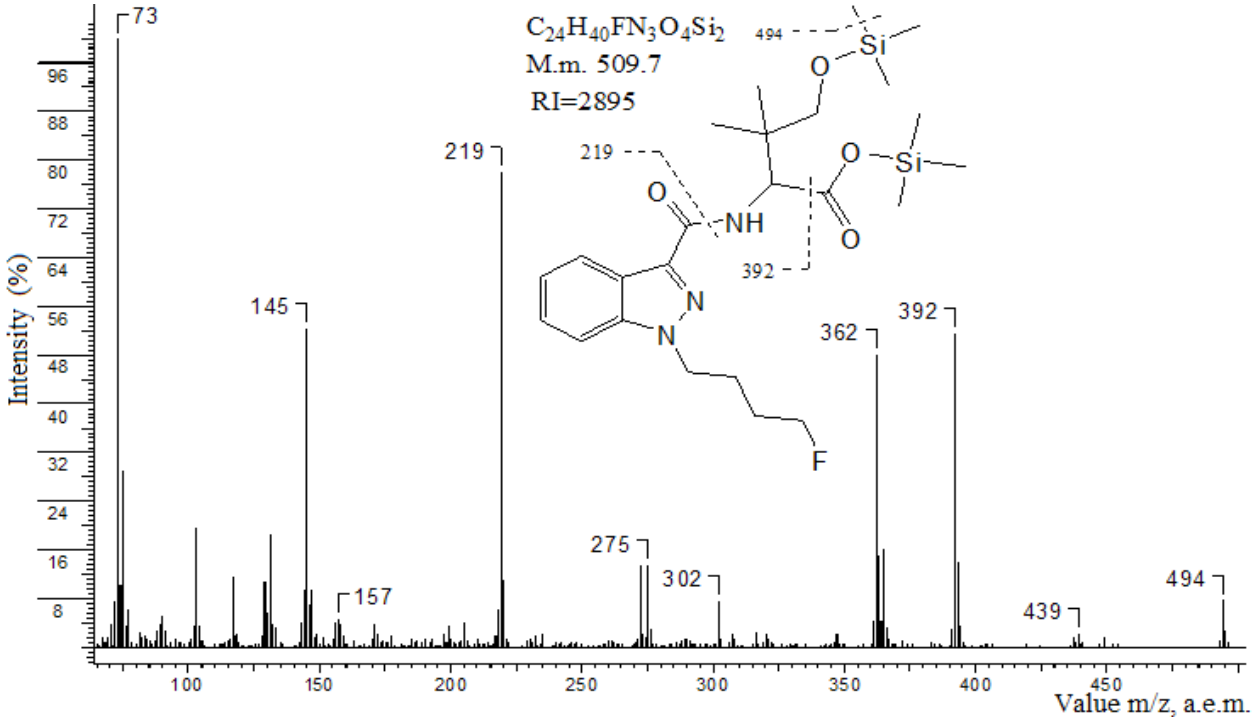


***Figure 16 – Mass-spectrum, retention index and structure of bis-trimethylsilyl ether of M4 Metabolite***

As a result of the research of the samples using sequential derivatization by methylation and subsequent acetylation or silylation for M6 and M8 metabolites, a shift in retention time and a change in the nature of mass fragmentation were observed. These factors indicate the presence of alcohol hydroxyl groups in the structure. As the resulting derivatives have a tendency to intramolecular cyclization with the formation of the corresponding artifact during gas chromatographic research, the relative content and degree of conjugation for M4 and M10 metabolites were determined in total after the preparation of methyl derivatives. The content of M10 was determined as an individual compound only after hydrolysis and derivatization with BSTFA.

In the mass spectra of methyl esters of MDMB(N)-073F metabolites, an expressed molecular radical ion is observed. There are general directions of fragmentation typical for methyl esters, with the exception of metabolite M7 and the artifact of metabolite M4, such as [M-59]+ and the cleavage of 2-methylprop-1-ene, generated from the tert-butyl group, forming radical ion [M-56]+.

Common characteristic ions for metabolites M1, M4 – M8 with m/z values of 131, 145, are shown in Fig. 17. For the dimethyl ester of metabolites M2.1, M2.2 (the presence of two isomers is due to the location of the hydroxyl group in the heterocycle of metabolites), there are expressed ions with m/z values of 161 and 175. Hereby the ions with m/z values of 131 and 145 are absent from the mass spectrum.

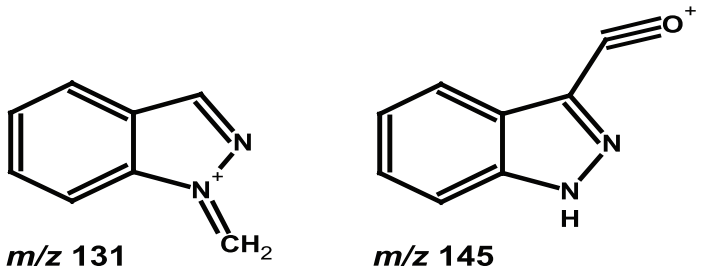


***Figure 17 – A supposed structure of characteristic ions peculiar to mass fragmentation of MDMB(N)-073F metabolites***

A high degree of cannabimimetic markers conjugation requires performing hydrolysis before their analysis (optimally: enzymatic or alkaline), while lipophilicity of markers makes it possible to extract them using hydrophobic or mixed-type sorbents (a combination of reversed-phase properties and cation exchanger properties). The latter make it possible to define SC markers directly in the urine screening procedure for narcotic and medicinal substances [5].

The use of SPE in the sample preparation made it possible to perform fractionation of substances into substances of acidic and alkaline nature. All identified MDMB(N)-073F metabolites were detected in eluate I.

The calculations of physicochemical constants LogP and $K_{os}$, the results of determining the degree of conjugation, the relative content of MDMB(N)-073F cannabimimetic and its metabolites in the studied urine samples are shown in the table 1.

A study of ten urine samples of MDMB(N)-073F cannabimimetics consumers showed that the majority of metabolites are excreted from the body in conjugated form. Unchanged cannabimimetic MDMB(N)-073F was not detected in the examined urine samples.

As it follows from the relative content of metabolites in the urine samples, the main metabolite of MDMB(N)-073F cannabimimetic is M1, which is the product of MDMB(N)-073F ester bond hydrolysis. Due to the expressed nature of the studied objects, metabolite M1 can be used as a marker of cannabimimetic MDMB(N)-073F.

Metabolites M10, M4 and their derivatives are the removable and form an artifact during the GC / MS study due to intramolecular cyclization (Fig. 6).

Metabolites M8 and M9 are common for MDMB(N)-073F and MDMB(N)-073 cannabimimetics [2]. The only identified MDMB(N)-073F metabolite with preservation of the ester bond at the level of sensitivity of the applied methods, was metabolite M10. The latter was identified in five urine samples with a relative content from 0.82% to 8.00% (median 2.72%). Other MDMB(N)-073F metabolites have no diagnostic value due to their low content in urine.

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*Table 1 – Characteristic of MDMB(N)-073F cannabimimetic and its major metabolites*

| Compound | Log P | Kos (pH=4.8) | Conjugation | | Relative content* | | |
|---|---|---|---|---|---|---|---|
| | | | n | (median, %) | Interval (n=10), % | n | median, % |
| MDMB(N)-073F | 2.89 | 893.54 | 10 | n.d.. | n.d. | – | – |
| M1 | 2.39 | 19.05 | 10 | 97.5 | 100 | – | – |
| M2.1 | 1.65 | 6.05 | 10 | 100 | 0.13 – 2.14 | 10 | 0.37 |
| M2.2 | 1.65 | 6.05 | 10 | 71.7 | 0.14 – 0.68 | 10 | 0.23 |
| M3 | 1.29 | 4.70 | 10 | 29.0 | 2.96 – 14.80 | 10 | 6.67 |
| M4** | 1.44 | 3.74 | 10 | 26.3 | 1.66 – 10.11 | 10 | 5.18 |
| M5 | 1.32 | 8.88 | 10 | 52.0 | 1.47 – 12.77 | 10 | 6.14 |
| M6*** | 1.13–1.28 | 3.90–4.57 | 10 | 85.7 | 0.18 – 1.33 | 10 | 0.51 |
| M7 | 2.39 | 27.04 | 9 | 83.7 | 0.07 – 0.98 | 9 | 0.49 |
| M8 | 1.23 | 4.51 | 5 | 100 | 0.14 – 0.75 | 5 | 0.36 |
| M9 | 0.55 | 1.66 | 10 | 83.1 | 0.11 – 6.06 | 10 | 0.36 |
| M10 | 2.09 | 324.71 | 5 | n.c. | 0,82 – 8,00 | 5 | 2.72 |

*Note:*

** The content of M1 is taken as 100%, the relative value of other metabolites was calculated according to the ratio of the areas of chromatographic peaks, formed by the most intense ions in their spectra. N.d. – not detected, n.c. – not calculated*

*** The relative content and conjugation were determined in total for metabolites M4 and M10.*

**** The values of LogP and $K_{os}$ vary depending on the location of the hydroxyl group in the alkyl chain.*

## CONCLUSIONS

On the basis of the gas chromatography-mass spectrometry method, its main metabolites have been identified in the urine samples of MDMB(N)-073F consumers. The physicochemical parameters have been calculated, mass spectral and chromatographic characteristics of some derivatives of the main MDMB(N)-073F metabolites have been obtained. The main metabolic pathways of MDMB(N)-073F have been defined; most of the produced metabolites are excreted in the urine as conjugates.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# STUDY OF RAPITALAM INFLUENCE ON OXOTREMORINE-INDUCED TREMOR

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*Parkinson's disease is the second most common (after Alzheimer's) neurodegenerative disease. All over the world, there is a search for new drugs aimed at the treatment of Parkinson's disease. Till up to the present, there is no "ideal" medicine that can completely cure this disease and has minimal adverse side effects. Belgorod research institute of pharmacology of living systems is studying Rapitalam, a new drug for the treatment of tremulous Parkinson's disease. This is an agonist of the mGluR4 group of metabotropic receptors.*

***The aim** of the article is to study Rapitalam influence on the oxotremorine-induced tremor in rats.*

***Methods.** The study comprised 60 rats (6 groups of 10 males), which were administered intragastrically with the studied substances for 10 days. All the animal groups except Control group 1, were administered with Rapitalam and the reference drug Levodopa. 30 minutes after Rapitalam and Levodopa, they were administered abdominally with the solution of Oxotremorine at the dose of 1.5 mg/kg. The animals of Control group 1, instead of Oxotremorine, were similarly administered with a solvent of 0.9% sodium chloride in the equivalent volume.*

***Results.** In comparison with the reference group, Rapitalam at the dose of 3 mg/kg significantly reduced the severity of tremor 50 min. after its administration. The same effect took place 30 min after the administration of Oxotremorine at the dose of 10 mg/kg. At the dose of 3 and 10 mg/kg, Rapitalam also decreased the number of rats in the group (in %) with the signs of tremor 60 min. and 50 min. after the administration of Oxotremorine, respectively.*

***Conclusion.** The study revealed that Rapitalam has a pronounced anti-tremor effect. Its administration at the studied doses reduced the symptoms of Oxotremorine-induced tremor in rats.*

***Keywords**: Parkinson's Disease; mGluR4; Rapitalam; Oxotremorine-induced tremor; Levodopa*

# ИЗУЧЕНИЕ ВЛИЯНИЯ РАПИТАЛАМА НА ОКСОТРЕМОРИН-ИНДУЦИРОВАННЫЙ ТРЕМОР

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***Аннотация.** Во всем мире идет поиск новых препаратов для лечения болезни Паркинсона, которая является вторым по распространенности нейродегенеративным заболеванием после болезни Альцгеймера. До настоящего времени «идеального» лекарственного средства, обладающего минимальными побочными эффектами и способного полностью вылечить пациентов с данным заболеванием, не существует. В научно-исследовательском институте «Фармакология живых систем» (г. Белгород) изучается новое лекарственное средство для лечения дрожательной формы болезни Паркинсона – Рапиталам, механизм действия которого заключается в активации метаботропных рецепторов группы mGluR4.*

***Цель** – изучить влияние Рапиталама на оксотреморин-индуцированный тремор у крыс.*

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ОРИГИНАЛЬНАЯ СТАТЬЯ
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***Методы.*** *В исследование было включено 60 крыс мужского пола, которые были разделены на 6 групп по 10 особей. Животным внутрижелудочно в течение 10 дней вводили исследуемые соединения. Всем группам животных, кроме группы Контроль 1, через 30 мин после введения Рапиталама и препарата сравнения Леводопа внутрибрюшинно вводили раствор оксотреморина в дозе 1,5 мг/кг. Животным из группы Контроль 1 аналогичным образом вместо оксотреморина вводили растворитель (0,9% раствор натрия хлорида) эквивалентном объеме.*
*Результаты. Введение крысам исследуемого соединения Рапиталам в дозе 3 мг/кг вызывало достоверное в сравнении с группой контроля снижение выраженности тремора через 50 мин, в дозе 10 мг/кг уже через 30 мин после введения оксотреморина. Также введение Рапиталама в дозе 3 и 10 мг/кг приводило к уменьшению количества крыс (выраженное в %) в группе с проявлениями тремора через 60 мин и 50 мин соответственно.*
***Заключение.*** *В результате исследования выявлено, что Рапиталам обладает выраженным антитреморным эффектом, что подтверждалось снижением проявлений оксотреморин-индуцированного тремора у крыс после введения исследуемого препарата.*
***Ключевые слова:*** *болезнь Паркинсона, mGluR4, Рапиталам, оксотреморин-индуцированный тремор, Леводопа*

## INTRODUCTION

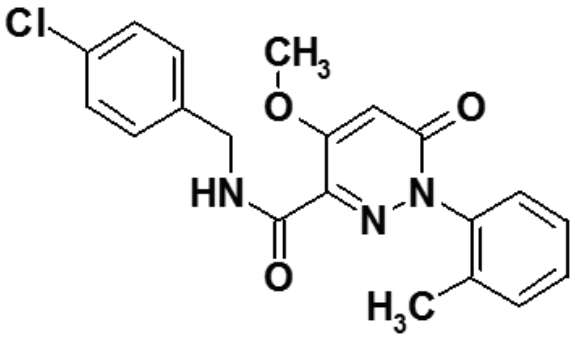
Parkinson's disease is a chronic disease characterized by dysfunction of the nervous system and movement disorders. The disease is progressive, that's why it is necessary to diagnose it as soon as possible and choose the necessary therapy. Drugs of different groups are selected individually in accordance with clinical manifestations of the disease. However, when prescribing the drugs, the problem of drug habituation and, consequently, necessity for dose escalation exists [1]. For this reason, at the early stages, doctors prescribe more sparing drugs in minimal doses. Unfortunately, there are none of the drugs for the treatment of Parkinson's disease which can completely cure this pathology. That is why the discovery of new therapeutic targets and drugs that can slow down the progression of neurodegeneration, remains an urgent task [2].

Metabotropic receptors (mGluRs) can have a neuromodulatory effect on both glutamatergic and GABA-ergic neurotransmission. That is very interesting for the development of new mGluR ligands which can be used to treatment of various neurological and mental disorders [3-5]. Metabotropic receptors mGLuR4 have been paid a lot of attention as a therapeutic target [6-8]. According to the literature data, a great number of mGLuR4 are presented in the synapses of striapallidal paths, in particular, in the neurons of the internal Globus pallidus segment and pars reticulate of the Substantia nigra [9].

Activation of these receptors leads to the reinforcement of GABA-ergic inhibition of the thalamus glutamatergic neurons by the striapallidal system, and consequently, to the correction of the imbalance between inhibitory and excitatory effects, shifted towards excitation, which is the basis of the pathogenesis of the tremulous form of Parkinson's disease [10, 11]. Previously, the mode of action of a new pharmacological substance – Rapitalam, was studied in Belgorod Research institute of pharmacology of living systems

The result of this study revealed that Rapitalam is an agonist of mGluR4 [2, 12].

**THE AIM** of the article is to study Rapitalam influence on the Oxotremorine-induced tremor in rats.




***Structure of Rapitalam.***

## MATERIALS AND METHODS

### *Animals*

The study was performed on male rats of Sprague Dawley line at 12-14 weeks of age, weighing 230-260 grams, obtained from the nursery of laboratory animals of "The Branch of the Institute of Bioorganic chemistry n.a. M.M. Shemyakin and Y.A. Ovchinnikov", Pushchino. All the animals were divided into 6 groups of 10 individuals (Table 1). The groups were formed at random using body weight as the leading feature so that the individual weight value hadn't deviated from the average value by more than 20%. Keeping the animals complied with all the rules of laboratory practice during preclinical studies in Russia. The animals were kept in the standard conditions corresponding to sanitary rules of SP 2.2.1.3218-14 "Sanitary and epidemiological requirements to the device, the equipment and the maintenance of experimental biological clinics (vivariums)" dated 29.08.2014 No 51. and GOST 33215-2014. The contents and all animal manipulations complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes (Strasbourg, 1986).

### *Study Design*

The test compound Rapitalam and the reference drug Levodopa, were administered to the animals intragastrically (per os) once a day for 10 days. The body weight of the animals was recorded just before each administration of the test and reference drugs.

***Table 1 – Animal groups***

| Group No | Administrated drug | Number of rats |
|---|---|---|
| 1 | Control 1 (0.5% tween-80 per os, 0.9% sodium chloride intraperitoneally) | 10 |
| | Oxotremorine-induced tremor | |
| 2 | Control 2 (0.5% tween-80 per os, oxotremorine intraperitoneally) | 10 |
| 3 | Levodopa 50 mg/kg | 10 |
| 4 | Rapitalam, 1 mg/kg | 10 |
| 5 | Rapitalam, 3 mg/kg | 10 |
| 6 | Rapitalam, 10 mg/kg | 10 |

*Note: PO – intragastrically, IP – abdominally*

### *Model of Oxotremorine-induced tremor*

All the animal groups except Control group 1, were injected intraperitoneally with an Oxotremorine solution at the dose of 1.5 mg/kg 30 min after the administration of the test compound and Levodopa. The animals of Control group 1 were similarly administered with 0.9% sodium chloride in the equivalent volume. The severity of Oxotremorine-induced tremor (in points) and the time of the symptoms reduction in rats were recorded.

### *Methods of statistical data analysis*

The intergroup *statistical* comparison was performed using the Kruskal-Wallis test with post-hocDunn test. For comparison of repeated measurements (Oxotremorin-induced tremor during the observation period), Repeatedmeasures ANOVA was used, in case of differences between the groups, the Bonferroni correction was used. The differences were determined at 0.05 significance value (GraphPad Prism 5.0).

## RESULTS

The onset of the tremor was observed in the animals of all the groups, which had been administered with Oxotremorine (Table 2).

***Table 2 – Latent period and tremor duration in groups of rats, M±m***

| | Latent period, min. | The tremor duration, min. |
|---|---|---|
| Control 1 | 0±0 | 0±0 |
| Control 2 | 10±0* | 68.8±5.8* |
| Levodopa 50 mg/kg | 10±0* | 62.2±4* |
| Rapitalam 1 mg/kg | 10±0* | 67.8±5.2* |
| Rapitalam 3 mg/kg | 10±0* | 57.8±2.8* |
| Rapitalam 10 mg/kg | 8.9±1.1* | 52.2±3.6*# |

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2 (non-parametric Kruskal-Wallis test, post-hocDunn test).*

Oxotremorine caused the increasing severity of tremor from 10-th to 20-th minutes after intra-abdominal injection to rats of Control group 2. Forty minutes after the administration of Oxotremorine the severity of tremor in the control rats began declining, and the tremor signs were not recorded beginning with 80-th min (Table 3).

In Group 3 administered with Levodopa at the dose of 50 mg/kg, there was a significant decrease in the severity of tremor 20, 30 and 50 minutes after Oxotremorine administration in comparison with Control group 2 (Table 3). A significant decrease in the % of rats with tremor in this group was registered 60 minutes after Oxotremorine administration (Table 4).

As Table 3 shows, the administration of Rapitalam at the dose of 1 mg/kg had no effect on the performance of tremor in rats. The Rapitalam doses of 3 and 10 mg/kg significantly decreased the severity of tremor. In comparison with the control group, the severity of tremor significantly decreased to 50-th min after the administration of Oxotremorine, at the dose of 3 mg/kg, and after the administration of Oxotremorine at the dose of 10 mg/kg, it significantly decreased beginning with 30-th min. Table 4 shows that Rapitalam at the doses of 3 and 10 mg/kg decreased the percentage of rats with tremor, 50 and 60 min. after the administration of Oxotremorine, respectively.

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***Table 3 – Points of severity of Oxotremorine-induced tremor in rats, M±m***

| Group | Time after Oxotremorine administration, min. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 |
| Control 1 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Control 2 | 1.8±0.3* | 2±0.3* | 1.9±0.4* | 1.5±0.3* | 1.3±0.3* | 0.6±0.3* | 0.4±0.2* | 0.1±0.1* | 0±0 | 0±0 | 0±0 | 0±0 |
| Levodopa 50 mg/kg | 1.4±0.2* | 1.2±0.3*# | 1±0.2*# | 1.1±0.1* | 0.6±0.2*# | 0.1±0.1* | 0.1±0.1* | 0.1±0.1* | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 1 mg/kg | 1.9±0.3* | 1.9±0.2* | 1.4±0.2* | 1.3±0.2* | 0.9±0.1* | 0.6±0.2* | 0.2±0.1* | 0.2±0.1* | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 3 mg/kg | 1.8±0.2* | 1.8±0.2* | 1.4±0.3* | 1.2±0.1* | 0.6±0.2*# | 0.2±0.1* | 0±0* | 0±0* | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 10 mg/kg | 1.3±0.3* | 1.4±0.2* | 1±0.2* | 0.8±0.1* | 0.2±0.1*# | 0.2±0.1* | 0±0*# | 0±0* | 0±0 | 0±0 | 0±0 | 0±0 |

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2 (Repeated measures ANOVA, Bonferroni correction).*

***Table 4. – Percentage of severity of Oxotremorine-induced tremor in rats, M±m***

| Group | Time after Oxotremorine administration, min | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 |
| Control 1 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Control 2 | 100±0* | 100±0* | 88.9±11.1* | 87.5±12.5* | 87.5±12.5* | 50±18.9* | 37.5±18.3* | 12.5±12.5* | 0±0 | 0±0 | 0±0 | 0±0 |
| Levodopa 50 mg/kg | 100±0* | 100±0* | 100±0* | 100±0* | 55.6±17.6* | 11.1±11.1*# | 11.1±11.1* | 11.1±11.1* | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 1 mg/kg | 100±0* | 100±0* | 100±0* | 88.9±11.1* | 88.9±11.1*& | 55.6±17.6*& | 22.2±14.7* | 22.2±14.7* | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 3 mg/kg | 100±0* | 100±0* | 88.9±11.1* | 100±0* | 55.6±17.6* | 22.2±14.7* | 0±0# | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Rapitalam 10 mg/kg | 88.9±11.1* | 100±0* | 77.8±14.7* | 77.8±14.7* | 22.2±14.7*#& | 22.2±14.7* | 0±0# | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2, &p<0.05 in comparison with L-DOPA 50 mg/kg (Repeatedmeasures ANOVA, Bonferroni correction)*

Thus, the effectiveness of Rapitalam with intragastric administration for 10 days (once a day) at the doses of 3 and 10 mg/kg was shown in the model of Oxotremorine-induced tremor in rats. The test compound at the dose of 3 mg/kg caused a significant decrease in the severity of the tremor from since 50-th min. and reduced the number of rats with tremors from 60 min. after Oxotremorine administration in comparison with the control. At the dose of 10 mg/kg Rapitalamat decreased the severity of tremor in the rats since 30-th min., and the number of rats in the group with the tremor signs since 50-th min. and further on. In the group of rats which were administered with the reference drug Levodopa at the dose of 50 mg/kg, the severity of tremor was significantly reduced since 50-th min. after Oxotremorine administration, and a significant decrease in the % of rats with tremorsin this groupwas observed 60 min. after Oxotremorine administration.

## DISCUSSION

Rapitalam decreased the severity of Oxotremorine-induced tremor in rats due to the pronounced anticholinergic activity. To explain the anticholinergic activity of Rapitalam, it is necessary to consider the interaction mechanisms in the extrapyramidal system of the brain in detail. The extrapyramidal system is a set of brain structures involved in the management of movements, maintenance of muscle tone and posture. This system can be represented in three major formations: paleostriatum (globus pallidus), neostriatum (caudate nucleus and putamen) substantia nigra [13]. With the normal functioning of the extrapyramidal system, cholinergic motor neurons of the spinal cord are in a state of constant activity and increase muscle tone. GABAergic neurons of globus pallidus inhibit motor neurons of the spinal cord, which leads to a decrease in the muscle tone. In parallel, GABAergic neurons of the caudate nucleus inhibit globus pallidus, therefore, inhibition of motor neurons of the spinal cord stops, and the muscle tone increases. The adequate control of the muscle tone in the extrapyramidal system is carried out by the interaction of excitatory glutamatergic neurons of the cortex, inhibitory dopaminergic neurons of substantia nigra and excitatory cholinergic neurons of the caudate nucleus (Fig. 1) [14, 15].

The pathogenesis of Parkinson's disease is based on

the death of dopaminergic neurons of substantia nigra, which leads to the increase in the tone of cholinergic neurons of the caudate nucleus. Under the influence of glutamatergic neurons of the cortex, stimulation of the cholinergic neurons of the caudate nucleus occurs and those, in turn, support GABAergic neurons in the active state. Since the caudate nucleus constantly inhibits globus pallidus, the latter does not have an inhibitory effect on motor neurons and the muscle tone remains high [16].

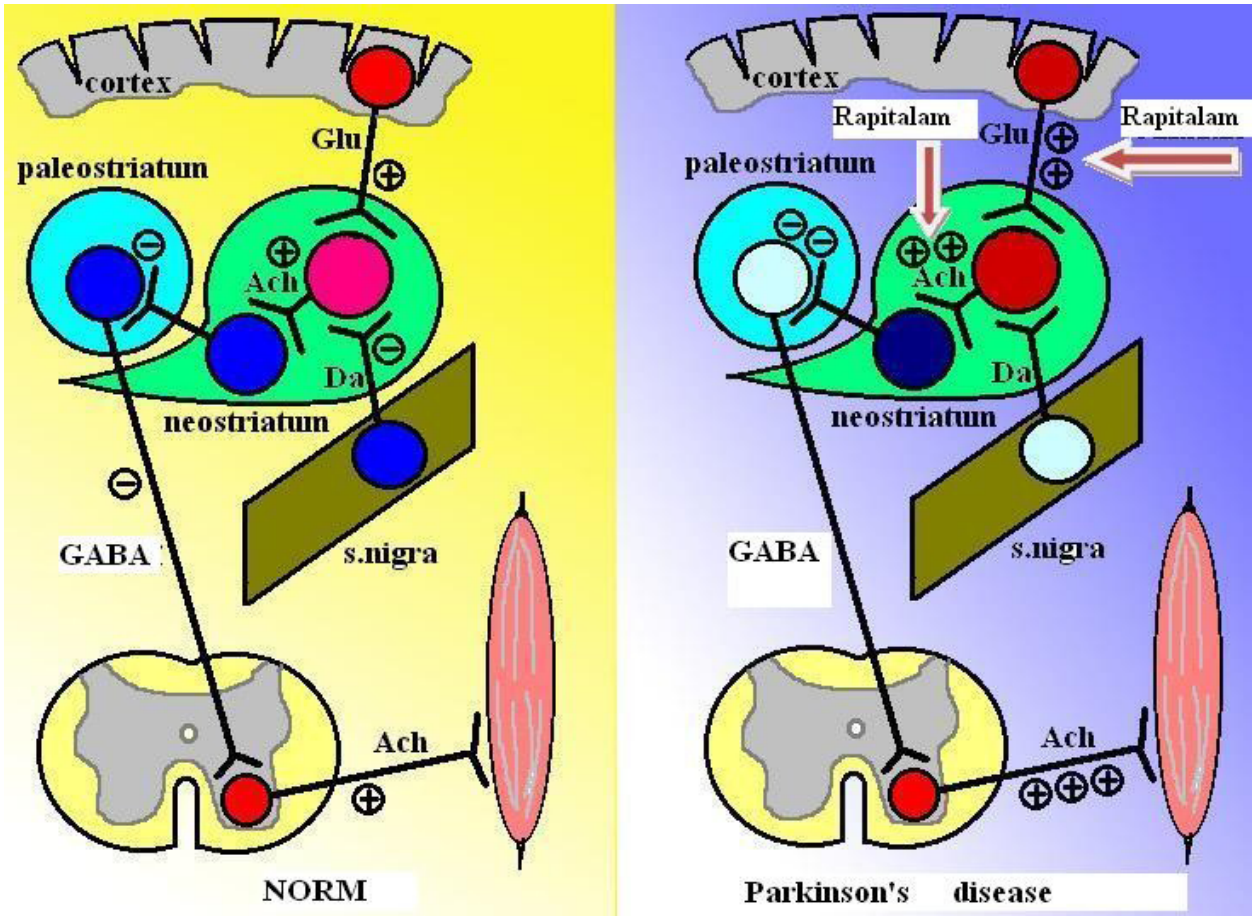


***Figure 1 – Biological targets of Rapitalam in Parkinson's disease [16].***

*Note: The normal relationships between the components of the extrapyramidal system are on the left. The picture that takes place in a patient with Parkinson's disease is on the right).*

*Ach – acetylcholine, Glu – glutamic acid, Da – dopamine.*

As Fig. 1 shows, Rapitalam suppresses glutamatergic neurons of the cortex and therefore inhibits cholinergic neurons of the neostriatumdue to the reduced stimulating effect of the thalamus. [17, 18]. Thus, under the influence of Rapitalam, there is a decrease in the acetylcholine level, as 1–2% of striatum neurons are represented by acetylcholine–containing interneurons [19], which causes an anticholinergic action of Rapitalam.

In this regard, in the long term, Rapitalam can solve the problem of treating tremor in Parkinson's disease at its early stages.

## CONCLUSION

At the dose of 1 mg/kg, Rapitalam does not have a significant effect on the manifestations of tremor in rats. Rapitalam (3 and 10 mg/kg) and Levodopa (50 mg/kg) have a comparable anti-tremor efficacy in rats. Rapitalam at the dose of 10 mg/kg reduces the symptoms of Oxotremorine-induced tremor in comparison with the control group in a shorter period of time than Levodopa.

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## CONFLICT OF INTEREST

The author declare no conflict of interest.

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# EFFECT OF FATTY OIL EXTRACT FROM SEEDS OF NIGELLA DAMASCENA L. ON LIPID SPECTRUM IN RATS WITH SIMULATED DYSLIPIDEMIA

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***The aim*** *of the study is to determine a lipid spectrum of blood plasma and liver in rats in with simulated dyslipidemia against the background of the administration of the fatty oil extract from the seeds of Nigella damascena L.*
***Materials and methods.*** *Laboratory animals – Wistar male rats – were used in the work. To study the hypolipidemic activity, such models as acute Tween, subchronic vitamin-D2 models and a model of chronic heart failure were used. The identifiable parameters were the concentration of cholesterol and triglycerides in the blood serum and liver, as well as the concentration of atherogenic and non-atherogenic lipoproteins in the blood serum, and the atherogenic coefficient.*
***Results.*** *As a result of the study, it was found out that a course administration of the fatty oil extract from the seeds of Nigella damascena L. against the background of simulated chronic heart failure (CHF) by the right ventricular type, normalizes the lipid spectrum of the experimental animals' blood serum, causing an increase in the concentration of high-density (non-atherogenic) lipoproteins, and reduces the concentration of low-density (atherogenic) lipoproteins. A single administration of the fatty oil extract from the seeds of Nigella damascena L. promotes the correction of lipid metabolism disorders under the conditions of acute Tween lipidopathy, while the direction of the object being studied reduces the concentration of cholesterol and triglycerides in the liver and blood serum under the conditions of subchronic dyslipidemia. At the same time, the effect of the use of the fatty oil extract from the seeds of Nigella damascena L. was not inferior to "Omacor", the reference drug.*
***Conclusion.*** *The possibility of using a fatty oil extract from the seeds of Nigella damascena L. for preventive and therapeutic aims in cardiovascular diseases has been established.*
***Keywords:*** *dyslipidemia, Nigella damascena L., Omacor, cholesterol, chronic heart failure*

# ВЛИЯНИЕ ЭКСТРАКТА ЖИРНОГО МАСЛА ИЗ СЕМЯН ЧЕРНУШКИ ДАМАССКОЙ (NIGELLA DAMASCENA L.) НА ЛИПИДНЫЙ СПЕКТР КРЫС ПРИ МОДЕЛИРОВАННОЙ ДИСЛИПИДЕМИИ

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***Цель исследования.*** *Провести исследование по определению липидного спектра плазмы крови и печени у крыс при моделированной дислипидемии на фоне введения экстракта жирного масла из семян чернушки дамасской.*
***Материалы и методы.*** *В работе использовались лабораторные животные – крысы самцы линии Wistar. Для изучения гиполипидемической активности использовали такие модели как – острую твиновую, субхроническую $Д_2$-витаминную модели и модель хронической сердечной недостаточности. Определяемыми параметрами служили концентра-*

**Для цитирования:** М.П. Ефремова. Влияние экстракта жирного масла из семян чернушки дамасской (nigella damascena l.) на липидный спектр крыс при моделированной дислипидемии. *Фармация и фармакология.* 2019;7(2): 90-96. **DOI:** 10.19163/2307-9266-2019-7-2-90-96



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*ция холестерина и триглицеридов в сыворотке крови и печени, а также концентрация атерогенных и неатнрогенных липопротеидов в сыворотке крови, и коэффициент атерогенности.*
***Результаты.*** *В результате исследования было установлено, что курсовое введение экстракта жирного масла из семян чернушки дамасской на фоне моделированной хронической сердечной недостаточности (ХСН) по правожелудочковому типу, нормализует липидный спектр сыворотки крови экспериментальных животных, вызывая увеличение концентрации липопротеидов высокой плотности (не атерогенных), и снижает концентрацию липопротеидов низкой плотности (атерогенных). Однократное введение экстракта жирного масла из семян чернушки дамасской способствует коррекции нарушений липидного обмена в условиях острой твиновой липидопатии, в то время как курсовое применение изучаемого объекта снижает концентрацию холестерина и триглицеридов печени, и сыворотки крови в условиях субхронической дислипидемии. При этом эффект от применения экстракта жирного масла из семян чернушки дамасской не уступал таковому у препарата сравнения «Омакор».*
***Заключение.*** *Возможность применения экстракта жирного масла из семян чернушки дамасской в профилактических и лечебных целях при сердечнососудистых патологиях.*
***Ключевые слова:*** *дислипидемия, чернушка дамасская, Омакор, холестерин, хроническая сердечная недостаточность*

## INTRODUCTION

Nowadays, cardiovascular diseases are the leading cause of mortality in both developed and economically developing countries. In many ways, a significant number of deaths are associated with a significant number of risk factors, which include dyslipidemia [1]. It has been established that lipid metabolism disorders play a significant role in the development of atherosclerosis and, as a consequence, coronary heart disease, myocardial infarction, ischemic stroke, chronic heart failure (CHF). That implies the need for correction of lipid imbalance [2].

The main lipid-lowering drugs include statins, fibrates and anion-exchange resins, which are recommended as one of the components of the treatment strategy, as well as a primary or secondary prevention of cardiovascular diseases in adults with a 20% or higher risk of developing pathologies of the cardiovascular system [3]. In large, randomized, controlled studies of statins, the use of statin drugs has been shown to reduce the risk of coronary heart disease and, in addition, to reduce overall mortality [4]. Therefore, these drugs are recommended as the first line of therapy, while fibrates and anion exchange resins are considered as the second line or means for combination therapy when statins are used [5]. However, despite the high efficiency, these drugs have a significant number of side effects limiting their use in patients with moderate increase of cholesterol and triglycerides in blood [6]. It was established that the composition of the extract of a fatty oil extract from the seeds of *Nigella Damascena* L. includes polyunsaturated fatty acids (oleic, eicosadiene, eicosan), amino acids, organic acids (myristic acid, benzoic acid), as well as tocopherol, β-sitosterol [9]. Thus, the rich chemical composition of a fatty oil extract from the seeds of *Nigella damascena* L. served as the basis for the inclusion of this object in the study. Alternative means of therapy in individuals with a moderate risk of atherogenesis may be drugs containing polyunsaturated fatty acids, which demonstrate a high level of efficacy and safety of use, both under experimental and clinical conditions [7, 8]. It has been established that a fatty oil extract from the seeds of *Nigella damascena* L. contains polyunsaturated fatty acids (oleic, eicosadienic, and eicosanoic), amino acids, organic acids (myristic acid, benzoic acid), as well as tocopherol, β-sitosterol [9]. Thus, the rich chemical composition of a fatty oil extract from the seeds of *Nigella damascena* L. has served the basis for the inclusion of this object in the study.

## MATERIALS AND METHODS

### *Biological model*

The experiment involved male Wistar rats weighing 220–240 grams in an amount of 150 individuals. The animals for the study had been grown in the vivarium of the Research Institute of Pharmacology Living Systems of Belgorod State University, Belgorod, and kept in a standard vivarium mode: the ambient temperature 22 ± 20°C, a 12-hour synchronized light mode; they were given combined food and water ad libitum. The experiment on the laboratory animals was conducted on the basis of the guidelines of the generally accepted bioethical principles of "the three R's", as well as the provisions of the Helsinki Declaration of the World Medical Association (2000), and the principles of humane experimental equipment under the UFAW program [10, 11]. Lipid-lowering properties of the a fatty oil extract from the seeds of *Nigella Damascena* L. were studied on three models of lipidopathology: acute Tween, subchronic $D_2$-vitamin models and a model of chronic heart failure (CHF) by the right ventricular type, at single and course administrations of the studied object. Hereby, 5 equal experimental groups of the animals (n = 10) were formed for each of the experimental models of dyslipidemia. The first group was represented by intact animals. The second group was a group of negative control rats (NC) with replicated lipidopathology, but devoid of pharmacological support. The third and the fourth groups of the animals received the investigated substance at the dose of 2.3 ml/kg given as a single dose and as a course administration (prophylactically for 14 days), respectively. The fifth group of the animals was administered the reference drug "Omacor" at the dose of 2 ml/kg. The studied substance and the reference drug were administered per os.

### *Model of acute Tween dyslipidemia*

This model was reproduced by a single intraperitoneal injection of Tween 80 (250 mg/100 g animal weight in 1 ml of water for injection). 12 hours after the introduction of Tween 80, the rats were euthanized in the morning by the method of cervical dislocation. The blood serum and liver of the tested rats were taken for the study. [12].

### *Modeling subchronic D2-vitamin lipidopathology*

Subchronic hyperlipidemia was simulated by a course administration of vitamin – $D_2$ in conjunction with the daily administration of alimentary cholesterol and merkazolil to inhibit the metabolism for 4 days. On the

5th day, the animals were decapitated and the biomaterial was collected – the serum and liver [13].

### *Model of chronic heart failure*

By Pyatnitsky N.N. and Blinkov Yu.A. method (1970), chronic heart failure by the right ventricular type was simulated. Under hexenal anesthesia (100 mg/kg body weight intraperitoneally) the rats were fractionally injected silicone oil into each pleural cavity (1.5 ml/100 g weight) [14]. After 30 days, another 1 ml of oil was added per 100 g of rat weight to each pleural cavity. The studied extract was administered a day after the reinjection of silicone oil for 14 days [15].

### *Biomaterial sampling and sample preparation*

A standard set of reagents "Lahema" was used to identify the concentration of total cholesterol and triglycerides in the blood serum. In the liver, cholesterol concentration was evaluated by a colorimetric method based on the Lieberman-Burchard reaction. The extraction from the liver tissue was performed according to Kolmakov's method [13]. The content of triglycerides in the liver was determined after its extraction, similar to the extraction of cholesterol, using a standard set of reagents "Lahema" [16].

The determination of low density lipoprotein (LDL), very low-density lipoproteins (VLDL) and high density lipoproteins (HDL) in the blood serum was carried out by turbidimetric analysis of Burstein and Samai. The principle of the method is the following: low density lipoprotein (LDL), very low-density lipoproteins (VLDL) and high density lipoproteins (HDL) form a complex with heparin, which is deposited without denaturation in the presence of calcium chloride. The presence of LDL and VLDL indicates the degree of turbidity. The treated solutions were measured at CPK-2 with the wavelength of 720 nm [17]. The coefficient of plasma atherogenicity ($K_a$) was also calculated using the formula [18]:

$$Ka = \frac{TC - HDL}{HDL}$$, where

TC – total cholesterol,

HDL – high density lipoproteins

The determination of lipoprotein lipase (LPL) in serum was performed according to the method of Titz et al. The test serum was applied to the stabilized suspension of olive oil. The released fatty acids were titrated with sodium hydroxide solution. The results were expressed in lipase units (LUs) [19].

### *Methods of statistical analysis*

The data obtained were statistically processed in the Microsoft Excel Ver 9, 2000 computer program package. The results were presented as M ± SEM. Student's t-test was used to compare the groups of means [20].

## RESULTS

In the group of NC rats the under the conditions of acute Tween lipidopathology, an increase in the concentration of cholesterol and triglycerides in the blood serum was observed in relation to intact animals by 70.1% ($p < 0.001$) and 70.15% ($p < 0.001$), respectively, with an increase in cholesterol and triglycerides in the liver by 23.5% ($p < 0.001$) and 75% ($p < 0.001$), respectively. It was also found out that the use of the fatty oil extract from the seeds of *Nigella damascena* L. at the dose of 2.3 ml/kg had significant hypocholesterolemic and hypotriglyceridemic effects in single and course administrations under the conditions of acute lipidopathology. In comparison with the control group, a decrease of the cholesterol level in the serum was observed with a single injection of the studied substance by 23.32% ($p<0.001$) and by 42.46% ($p<0.001$) during a course administration. In the liver, in relation to the negative control group of rats, the cholesterol content decreased by 27.16% ($p<0.001$) with a single use of the studied substance. The contents of triglycerides in the blood serum and liver decreased (relative to the control group) by 57.46% ($p<0.001$) and 25.82% ($p<0.001$) after a single administration of the fatty oil extract from the seeds of *Nigella damascena* L. In the course of the administration of the studied substance, the contents of triglycerides in the liver and serum decreased in comparison with the control group of rats by 43.86% ($p < 0.001$) and 35.19% ($p < 0.001$), respectively. With the administration of "Omacor", the concentration of cholesterol and triglycerides in the blood serum of the animals decreased by 55.87% ($p<0.001$) and 44.74% ($p<0.001$), respectively. In the liver, these figures decreased by 47.65% ($p<0.001$) and 47.80% ($p<0.001$), respectively, relative to the control group.

***Table 1 – Changes in lipid metabolism in the blood serum and liver after the administration of fatty oil extract from the seeds of Nigella Damascena L. under the conditions of acute Tween dyslipidemia***

| Animal groups | Cholesterol, mmol/l Blood serum | Triglycerides, mmol/l Blood serum | Cholesterol, mg/g Liver | Triglycerides, μmol/g Liver |
|---|---|---|---|---|
| Intact animals | 4.21±0.14 | 1.34±0.01 | 3.28±0.23 | 1.04±0.01 |
| Negative control animals | 7.16±0.33# | 2.28±0.12# | 4.05±0.13# | 1.82±0.02# |
| Fatty oil extract from the seeds of *Nigella damascena* L. (single administration) | 5.49±0.25* | 0.97±0.02* | 2.95±0.15* | 1.35±0.23* |
| Fatty oil extract from the seeds of *Nigella damascena* L. (course administration) | 4.12±0.31* | 1.28±0.12 | 4.12±0.31# | 1.18±0.11* |
| "Omacor" | 3.16±0.21* | 1.26±0.11* | 2.12±0.05*# | 0.95±0.02* |

*Note: # – statistically significant relative to intact animals (p<0.001);*
** – statistically significant relative to the NC group of rats (p<0.001).*

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The influence of the lipoproteinlipase enzyme serum was also taken into account. The effect of the fatty oil extract from the seeds of *Nigella damascena* L. at the dose of 2.3 ml/kg on the lipoprotein lipase activity was determined in intact white rats and the rats with simulated Tween hyperlipidemia.

The experiments have shown (Table 2) that the activity of lipoprotein lipase in intact animals was 1.213±0.21 lipase units (LUs). Against the background of a single injection of a fatty oil extract from the seeds of *Nigella damascena* L. at the dose of 2.3 ml/kg, the enzyme activity was increased to 2.833±0.17 LUs, i.e., by 133.6%% ($p<0.001$). A slightly less pronounced effect was observed when a course administration of a fatty oil extract from the seeds of *Nigella damascena* L. to healthy animals was performed. The activity of LPL in these experiments increased to 2.45±0.24 LUs. This is 102% ($p<0.001$) higher than that of intact animals. In experimental hyperlipidemia in the group of control animals treated with physiological solution, there was an unreliable decrease in the activity of the enzyme compared with its value in intact animals ($p<0.05$). The increase in LPL activity in experimental animals with a simulated acute pathology after single and course administrations of the studied fatty oil extract from the seeds of *Nigella damascena* L. at the dose of 2.3 ml / kg amounted to 1.784±0.13 LUs and 1.321±0.20 LUs, respectively. It should be noted that, against the background of Tween intoxication, with a single injection of a fatty oil extract from the seeds of *Nigella damascena* L., the activity of LPL was higher than with a course administration. Against the background of the use of "Omacor", LPL activity increased relative to the control group by 123.1% ($p <0.001$) and by 64.1% ($p <0.001$) in comparison with the intact group of animals.

***Table 2 – Effect of fatty oil extract from the seeds of Nigella damascena L. at the dose of 2.3 ml/kg on LPL serum activity***

| Animal groups | LPL, LU | |
|---|---|---|
| | M±m | % , P |
| Intact animals | 1.213±0.21 | 100% |
| Fatty oil extract from the seeds of *Nigella damascena* L. (single administration), 2,3 ml/kg | 2.833±0.17 | +133.6% $p_1<0.001$ |
| Fatty oil extract from the seeds of *Nigella damascena* L. (course administration), 2,3 ml/kg | 2.45±0.24 | +102% $p_1<0.001$ |
| Physiological solution+Tween (control) | 0.892±0.25 | -26.5% $p_1>0.5$ |
| Fatty oil extract from the seeds of *Nigella damascena* L. (single administration), 2,3 ml/kg + Tween 80 | 1.784±0.13 | +47.07% $p_1<0.5$<br>+100% $p_2<0.001$ |
| Fatty oil extract from the seeds of *Nigella damascena* L. (course administration), 2,3 ml/kg + Tween 80 | 1.321±0,20 | +8.9% $p_1<0.001$<br>+48.1% $p_2<0.001$ |
| Omacor | 1.99±0.361 | +64.1% $p_1<0.001$<br>+123.1% $p_2<0.001$ |

*Note: $p_1$ – significance of differences with respect to indicators of intact animals;*
*$p_2$ – significance of differences with respect to indicators of control animals*

In the animals of the NC group, serum and liver cholesterol concentrations increased by 112.04% ($p <0.001$) and 85.6%, respectively, with subchronic D2-vitamin lipidopathology, while the serum and liver triglycerides increased 131.5% ($p <0.001$) and 211.5% ($p <0.001$), respectively. Under the conditions of vitamin-$D_2$ lipidopathology (Table 3), a course administration of a fatty oil extract from the seeds of *Nigella damascena* L. contributed to a decrease in serum cholesterol and triglycerides, compared to the NC group of rats, by 24.4% ($p<0.001$) and 25.73% ($p<0.001$), respectively. After a single administration of a fatty oil extract from the seeds of *Nigella damascena* L., the concentration of serum cholesterol and triglycerides did not change statistically significantly in comparison with the NC group of the animals. In the liver, the cholesterol content decreased in relation to the NC group of rats by 24.6% ($p<0.001$) and 22% ($p<0.001$), respectively, with a single and course administration of a fatty oil extract from the seeds of *Nigella damascena* L. In the liver, the concentration of triglycerides decreased only while the course administration of a fatty oil extract from the seeds of *Nigella damascena* L. – by 36.7% ($p<0.001$) relative to the animals of the NC group (Table 3). The use of "Omacor" contributed to the decrease in serum and liver cholesterol levels in comparison with the NC group of rats by 45.7% ($p<0.001$) and 43.5% ($p<0.001$), respectively, while the concentration of triglycerides decreased by 30.8% ($p<0.001$) and 32.7% ($p<0.001$), respectively.

***Table 3 – Changes in lipid metabolism in the blood serum and liver after the administration of a fatty oil extract from the seeds of Nigella damascena L. under the conditions of subchronic $D_2$-vitamin dyslipidemia***

| Animal groups | Cholesterol, mmol/l Blood serum | Triglycerides, mmol/l Blood serum | Cholesterol mg/g Liver | Triglycerides, μmol/g Liver |
|---|---|---|---|---|
| Intact animals | 3.57±0.12 | 1.78±0.01 | 3.26±0.13 | 1.04±0.01 |
| NC animals | 7.57±0.24# | 4.12±0.12# | 6.05±0.17# | 3.24±0.02# |
| Fatty oil extract from the seeds of *Nigella damascena* L. (single administration) | 6.78±0.15# | 4.23±0.02# | 4.56±0.31*# | 3.25±0.42# |
| Fatty oil extract from the seeds of *Nigella damascena* L. (course administration) | 5.72±0.52*# | 3.06±0.11*# | 4.72±0.37*# | 2.05±0.22*# |
| "Omacor" | 4.11±0.11* | 2.85±0.17*# | 3.42±0.43* | 2.18±0.41*# |

*Note: # – statistically significant relative to the intact animals (p<0.001);*
** – statistically significant relative to the NC group of rats (p<0.001)*

A study of the experimental rats' blood with simulated pathology of CHF showed a pronounced disorder in the lipid spectrum (Table 4). That was reflected in an increase in the atherogenic coefficient ($K_a$) by 111.32% (p <0.05), compared to the animal group not subjected to the experimental exposure. Dyslipidemia can be judged by the increased concentration of atherogenic lipoproteins – LDL – by 178.2%, and total cholesterol by 25.1% compared with the group of intact animals. A decrease in non-atherogenic lipoproteins by 19.11% relative to the intact group of rats was also observed.

It should be noted that in intact rats, the administration of the studied substance did not significantly affect the concentration of total cholesterol and lipoproteins.

Judging by the lipid profile (Table 4), it can be concluded that in case of a simulated pathology of CHF, a course administration of *Nigella damascena* L. at the dose of 2.3 ml/kg prevents the disturbance of lipid metabolism. The concentration of atherogenic lipoproteins (LDL) after the course administration of a fatty oil extract from the seeds of *Nigella damascena* L. has reduced by 39.5% (p <0.05) relative to the group of the NC animals. And the concentration of non-atherogenic lipoproteins, by contrast, increased by 23.5%, (p <0.05) relative to the negative control group of rats. In the course administration of a fatty oil extract from the seeds of *Nigella damascena* L., the total cholesterol had also a tendency to decrease by 13.65% (p <0.05). The atherogenic coefficient of blood plasma against the background of a course administration of a fatty oil extract from *Nigella damascena* L. decreased statistically significantly by 46.8% (p <0.05) compared with the control group of animals. Against the background of the administration of "Omacor" in the animals compared with the control group, there was an increase in HDL by 32.5% (p <0.05), as well as a decrease in TC, LDL and atherogenicity by 15.4% (p <0.05); 34.7% (p <0.05); 56.2% (p <0.05), respectively. At the same time, no statistically significant differences between the groups of rats treated with "Omacor" and *Nigella damascena* L. during the course and single administrations under the conditions of CHF model have been established.

***Table 4 – Effect of a fatty oil extract from the seeds of Nigella damascena L. at the dose of 2.3 ml/kg on lipid metabolism in rats with simulated CHF***

| Group | TC | HDL | LDL | VLDL | Ka |
|---|---|---|---|---|---|
| Intact | 1.833±0.08 | 1.015±0.06 | 0.234±0.03 | 0.583±0.04 | 0.848±0.11 |
| % | 100 | 100 | 100 | 100 | 100 |
| Control (pathology) | 2.293±0.02* | 0.821±0.03 | 0.651±0.05* | 0.569±0.03* | 1.792±0.12* |
| % to intact | +25.09 | –19.11 | +178.2 | –2.4 | +111.32 |
| Fatty oil extract from the seeds of *Nigella damascena* L., 2.3 ml/kg, without pathology | 1.762±0.021 | 0.989±0.027 | 0.146±0.053 | 0.582±0.031 | 0.782±0.03 |
| % to intact | –3.9 | +2.6 | –37.61 | –0.17 | –7.8 |
| Fatty oil extract from the seeds of *Nigella damascena* L., 2.3 ml/kg (single administration+ pathology) | 2.021±0.02 | 1.001±0.02 | 0.341±0.12# | 0.453±0.03 | 1.019±0.08 |
| % to control | –12 | +21.9 | –47.6 | –20.4 | –41.97 |
| Fatty oil extract from the seeds of *Nigella damascena* L., 2.3 ml/kg (course administration+ pathology) | 1.980±0.110# | 1.014±0.042# | 0.394±0.112#* | 0.572±0.010 | 0.953±0.06# |
| % to control | –13.65 | +23.51 | –39.47 | –1.89 | –43.1 |
| "Omacor" | 1.942±0.213# | 1.088±0.129# | 0.425±0.029#* | 0.523±0.098 | 0.509 ±0.143# |
| % to control | –15.4 | +32.5 | –34.7 | –8.1 | –56.2 |

*Note: * – to intact animals (p <0.05)*
*# – to the group of simulated pathology (control) (p <0.05)*

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## DISCUSSION

Thus, under the conditions of acute Tween lipidopathology, the most pronounced effect of lipid metabolism indices in the blood serum and liver was observed with a single administration of the studied extract of a fatty oil extract from the seeds of *Nigella damascena* L. It should be also noted that the extract of the fatty oil from the studied extract has an activating effect on the serum lipoprotein lipase in the acute Tween model of hyperlipidemia and without a simulated pathology, and in both – single and course – uses.

When simulating subchronic $D_2$-vitamin dyslipidemia, in the course administration of a fatty oil extract from the seeds of *Nigella Damascena* L., the positive dynamics of lipid metabolism, in the direction of lowering the level of cholesterol and triglycerides in the blood serum and liver, was observed compared with the negative control group.

In a simulated experimental pathology of CHF in rats, pathological changes in lipid metabolism were observed in increasing $K_a$ concentration, which is comparable with the described manifestations in the clinic [21,22]. In the course and single administrations of a fatty oil extract from the seeds of *Nigella Damascena* L., the concentration of atherogenic lipoproteins significantly decreased and the concentration of HDL in the serum increased, which is also confirmed by literature data [23].

Hereby it should be notified that the effect of the administration of the studied extract was comparable to that of the reference drug – "Omacor". The indicators of the animals treated with the fatty oil extract from the seeds of *Nigella damascena* L., did not significantly differ from the results of the group of the animals receiving "Omacor", the reference drug. It has been proven that the hypotriglyceridemic effect observed in our experiments while the administration of the investigated fatty oil extract from the seeds of *Nigella damascena* L. to animals is associated with the activation of the enzyme metabolizing fatty acids and triglycerides – LPL (lipoprotein lipase). The use of fatty oil extract from the seeds of *Nigella damascena* L. contributed to the restoration of lipid metabolism, expressed in the reduction of cholesterol and triglycerides in the blood serum and liver. A similar effect of the fatty oil extract is probably associated with its polyunsaturated fatty acids and timoquinone. The data on its lipid-correcting action are given in the literature [24].

## CONCLUSION

It follows from the experiment that single and course administrations of a fatty oil extract from the seeds of *Nigella damascena* L. affect lipid metabolism. This is reflected in a decrease in cholesterol and triglycerides in the blood and liver of the animals under the conditions of Tween and vitamin hyperlipidemia.

It has also been established that, against the background of simulated CHF, during the course administration of a fatty oil extract from the seeds of *Nigella damascena* L., the lipid spectrum normalizes, the concentration of high-density lipoproteins increases relative to the control group, and the concentration of low-density (atherogenic) lipoproteins significantly decreases. Under the conditions of experimental CHF, the administration of a fatty oil extract from the seeds of *Nigella damascena* L. decreases the atherogenic coefficient of blood plasma relative to the control group of the animals. The studies of the activity of serum lipoprotein lipase in single and course administrations of the fatty oil extract from the seeds of *Nigella damascena* L. indicate the activating effect on the LPL enzyme.

The extract of the fatty oil from the seeds of *Nigella damascena* L. has a pronounced hypolipidemic effect in animals with experimental hyperlipidemia, manifested in a decrease in LDL, cholesterol, serum triglycerides, and also cholesterol and triglycerides in liver. This effect is not inferior to the similar effect of the official lipid-lowering drug "Omakor".

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## CONFLICT OF INTEREST

The author declare no conflict of interest.

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*ОРИГИНАЛЬНАЯ СТАТЬЯ*
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# EXPERIMENTAL STUDY OF ANTI-THROMBOTIC ACTIVITY OF PENTOXYFILLIN MICROPARTICLES: BASED ON POLY-DL-LACTIDE-CO-GLYCOLIDE IN COMPARISON WITH PENTOXYFILLIN

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***The aim*** *of the work was a comparative experimental study of the effect of oral administration of Pentoxifylline microparticles based on PLGA, and "standard" Pentoxifylline, on the ADP-induced platelet aggregation process in rats.*

***Materials and methods.*** *Pentoxifylline substance (100 mg/kg) was used as a reference drug, and PLGA-based Pentoxifylline microparticles with an average dynamic radius of 175.4 nm were used as the object in study. In the experiment, male Wistar rats (m = 300–330 g), the same age group (9 months) were used. They were divided into 3 groups, each of 6 animals. The antiplatelet activity was assessed by determining the degree and rate of platelet aggregation in 1, 3, 5, 8 and 24 hours after a single oral administration of the reference drug and the object under study. Adenosine diphosphate (ADP) at the concentration of 5 μM was used as an aggregation inducer. The aggregation process was recorded using a two-channel laser platelet aggregation analyzer ALAT-2, wavelength of 0.785 μm. by determining the average conventional size of the aggregates.*

***Results.*** *The experiment has proved the following: PLGA-based Pentoxifylline microparticles are more effective at reducing the possibility of platelets to aggregate within 24 hours of the investigation (more than 40%) conventional to the control group value. Besides, it should be noted that according to the effectiveness of the pharmacological action during AD-induced platelet aggregation, the microparticles are commensurate with the standard sample - Pentoxifylline. The action of the microparticle object under study lasts for 24 hours, while the effect of the reference drug is over after 3 hours and then the indicators of the reference group do not differ from those of the control onel.*

***Conclusion.*** *When administered per os, PLGA-based Pentoxifylline microparticles prolong the pharmacological effect significantly – up to 24 hours.*

***Keywords:*** *Pentoxifylline, poly-DL-lactide-co-glycolide, Pentoxifylline microparticles, rheological properties of blood, antiplatelet agents*

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# ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ АНТИТРОМБОЦИТАРНОЙ АКТИВНОСТИ МИКРОЧАСТИЦ ПЕНТОКСИФИЛЛИНА НА ОСНОВЕ ПОЛИ-DL-ЛАКТИД-КО-ГЛИКОЛИДА В СРАВНЕНИИ С ПЕНТОКСИФИЛЛИНОМ

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***Цель*** *– сравнительное экспериментальное изучение влияния перорального введения микрочастиц пентоксифиллина на основе PLGA и «стандартного» пентоксифиллина, на процесс АДФ-индуцированной агрегации тромбоцитов крыс.*

***Материалы и методы.*** *В качестве препарата сравнения использовалась субстанция пентоксифиллина (100 мг/кг), в роли исследуемого объекта – микрочастицы пентоксифиллина на основе PLGA (100 мг/кг) со средним динамическим радиусом 175,4 нм. В эксперименте использовались крысы-самцы линии Wistar (m = 300–330 г), одной возрастной группы (9 месяцев), разделенные на 3 группы по 6 животных. Антиагрегантную активность оценивали путем определения степени и скорости агрегации тромбоцитов через 1, 3, 5, 8 и 24 часа после перорального однократного введения препарата сравнения и исследуемого объекта. Аденозин дифосфат (АДФ) в концентрации 5 мкМ применяли в роли индуктора агрегации. Процесс агрегации регистрировали с применением системы двухканального лазерного анализатора агрегации тромбоцитов «АЛАТ – 2», длина волны 0.785 мкм, методом определения среднего относительного размера агрегатов.*

***Результаты.*** *В ходе эксперимента было доказано следующее: микрочастицы пентоксифиллина на основе PLGA более эффективно уменьшают способность тромбоцитов к агрегации в течении 24 ч. исследования (больше, чем на 40%) относительно значений контрольной группы, кроме того следует отметить, что по эффективности фармакологического действия во время АДФ-индуцированной агрегации тромбоцитов микрочастицы соизмеримы со стандартным образцом – пентоксифиллином. Действие исследуемого объекта микрочастиц продолжается в течение 24 ч., в то время как действие препарата сравнения заканчивается через 3 часа и далее показатели группы сравнения не отличаются от показателей контроля.*

***Заключение.*** *Микрочастицы пентоксифиллина на основе PLGA при пероральном введении существенным образом пролонгируют фармакологическое действие до 24 ч.*

***Ключевые слова:*** *пентоксифиллин, поли-DL-лактид-ко-гликолид, микрочастицы пентоксифиллина, реологические свойства крови, антиагреганты*

## INTRODUCTION

In pathological conditions such as strokes and heart attacks, platelet thrombi play a triggering role. [1]. The task of pharmacy is the development and study of highly effective drugs that comprehensively affect vascular-platelet hemostasis [2–5]. In a number of products aimed at improving the rheological properties of blood, Pentoxifylline has been used most widely [6, 7]. In patients with complex cardiovascular pathology, Pentoxifylline has the most convincing basis for the correction of perfusion disorders [8, 9]. In cerebrovascular and peripheral vascular diseases of atherosclerotic genesis, Pentoxifylline is included in the treatment standards [10–13]. Its use is pathogenetically and clinically validated for the treatment of patients with systemic atherosclerosis [14–16].

Pentoxifylline is known to play the role of a weak P2Y-receptor antagonist, thus it competes with ADP for the ability to bind to these receptors, resulting in a decrease in the proaggregant effect of ADP on the purine receptors and the assembly process of integral receptors. Pentoxifylline helps to reduce platelet aggregation and adhesion, and also has a vasodilating effect. It also has a weak cardiotonic effect, caused by the process of blocking phosphodiesterase of type III in cardiomyocytes [1].

It should be also noted that Pentoxifylline reduces the process of fibrinogen synthesis, and reinforces the occurrence of tissue plasminogen activator (I-PA). This leads to the increased activity of the fibrinolytic system [1, 17–20].

The primary metabolism of Pentoxifylline occurs in the blood. Up to seven metabolites are formed during that process, two of them are characterized by a pronounced antiaggregant activity. The final metabolism of Pentoxifylline occurs in the liver [21].

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Pentoxifylline is characterized by good tolerance, due to this it is possible to combine its use with many other drugs. Pentoxifylline preparations existing on the pharmaceutical market today, require a triple administration per day, which makes the treatment process rather compliant. In addition, if a patient does not take the drug in time, there is not only a decrease in the effectiveness of therapy, but also a risk of an impairment increase of hemorheological blood properties. In this regard, the creation of Pentoxifylline with prolonged properties is relevant and promising [3, 22, 23].

According to the literature data, the use of prolonged forms based on PLGA (Somatulin, Sandostatin Lar and others), allows increasing the bioavailability of the drug and its delivery to the target organ, maintaining a constant therapeutic concentration in the blood and reducing the frequency of administration. The advantages of PLGA should also include the fact that it has low toxicity, and when ingested, it is completely biodegradable [23]. Considering the above, Pyatigorsk Medical and Pharmaceutical institute outlines the research to create an innovative, prolonged dosage form of Pentoxifylline based on PLGA.

***The aim*** of the work is to study the effect of oral administration of Pentoxifylline microparticles basing on poly-DL-lactide-co-glycolide, on ADP-induced aggregation process, in comparison with Pentoxifylline.

## MATERIALS AND METHODS

### *Animals*

The lab rats were obtained from the vivarium of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. Keeping experimental animals complied with the current regulatory documentation, i.e., the "Sanitary rules for the design, equipment, and maintenance of experimental biological clinics (vivariums)". The animals were kept on a standard diet that complies with current regulations. Feeding was carried out at a fixed time. For drinking, the laboratory animals were supplied with drinking bowls. The environmental factors (temperature, humidity, light intensity and air exchange rate, litter composition) met the requirements for keeping laboratory animals The cages, drinking bowls, litter were changed at least once a week. [24]. The contents and all animal manipulations complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes (Strasbourg, 1986).

### *Study design*

Pentoxifylline substance (100 mg / kg, "TCI", USA, Lot. BRDTB-FM, P 2050) was used as a reference drug, and PLGA-based Pentoxifylline microparticles with an average dynamic radius of 175.4 nm were used as the object under study. They were obtained on the base of Pyatigorsk Medical and Pharmaceutical Institute – branch Volgograd State Medical University. An optimal technology has been developed for obtaining a prolonged dosage form of Pentoxifylline based on PLGA, namely the ratio of Pentoxifylline and poly-DL-lactide-co-glycolide (50:50), mol.wt 40,000-75.000 (Sigma) – 1: 3. Accurate portions of the polymer and Pentoxifylline substance are dissolved in 2 ml of solvent (chloroform), then the finished composition is added dropwise to the aqueous solution of polyvinyl alcohol at the concentration of 0.3%. The process takes place with continuous operation of the homogenizer at 20,000 rpm for 15 minutes. The finished solution is centrifuged at 6000 rpm for 40 minutes, then the supernatant is decanted and returned for the subsequent analysis.

The recovered sediment of microparticles is washed with purified water. After that it is centrifuged again (4 times). The finished microparticles are transferred to a 25 ml flask, and brought to the mark with purified water. This dosage form is used for pharmacological studies [22, 23].

During the experiment, healthy adult male Wistar rats (m = 300–330 g) of the same age group (9 months), which had been quarantined for 14 days, were used.

With the help of the method of random sampling three groups of 6 animals were created:

– Group 1 – the animals which received a 0.9% sodium chloride solution *per os* in an equivalent volume (control group);

– Group 2 – the animals, which were given a single dose of Pentoxifylline at the dose of 100 mg/kg *per os* (experimental group);

– Group 3 – the animals, which were given a single dose of a prolonged form of Pentoxifylline at the dose of 100 mg/kg *per os* (experimental group).

The objects of the study were administrated at the fixed time of the day (8-00 - 8-30). Considering the fact that Pentoxifylline is widely used in clinical practice *per os* [25], this route of administration was used in the further study. For that, a suspension was prepared in a 0.9% solution of sodium chloride, which was then administered to the animals using a special probe in a volume of 10 ml/kg. The effect of the objects of the study on platelet aggregation was studied at the dose of 100 mg/kg (in terms of Pentoxifylline).

Based on the scientific data on effective therapeutic doses of Pentoxifylline, as well as taking into account the coefficient of conversion of the dose from human to rat, this dose was determined by calculation. [26, 27].

Blood sampling from the animals was carried out on an empty stomach in the morning. To prevent the blood clotting process, a 3.8% solution of sodium citrate was added at the ratio of 1:9. Silicone dishes were used to exclude a contact platelet activity. The induced platelet aggregation was investigated immediately after taking blood for analysis.

Platelet rich plasma (PRP) was obtained and platelet counts were calculated using the standard method [28, 26]. With the help of the centrifuging method (a PC-6

centrifuge was used in the experiment) at 400 g and 1800 g, respectively, PRP was obtained from the blood samples taken for the analysis.

In the Goryaev chamber platelet counts in PRP were performed with the use of the microscopic method with phase contrast. Normally, in the blood of a rat, the number of platelets varies widely - from 430,000 to 1 million in 1 $mm^3$ – after the analysis of the number of platelets in PRP. To analyze the platelet count of PRP, standardization of the platelet count was carried out, for which the PRP was diluted with the necessary number of PRP to 400 ± 30 thousand platelets in 1 $mm^3$ in the sample.

### *Defined indicators*

The antiplatelet activity of the prolonged form of Pentoxifylline was evaluated by the degree of platelet aggregation. The indices were recorded after 1, 3, 5, 8 and 24 hours after a single administration of Pentoxifylline microparticles based on poly-DL-lactide-co-glycolide. ADF ADP (NPO "RENAM", Russia) acted as an inducer of aggregation with a total concentration of 5 μM [26].

By laser aggregometry, platelet aggregates and a detection and determination of their sizes were held. An assessment of the degree of dispersion of the light beam and fluctuations in the analysis of the optical density were carried out taking into account the light transmittance curve and the size of the aggregates.

This method allows to investigate the platelet aggregation process, size and shape of aggregates. When adding an inductor, the degree of aggregation has a maximum value of the average size of the units [29, 30]. According to the obtained aggregatogram the extent of platelet aggregation was determined.

The conditions in the study of platelets on the aggregometer were close to physiological, namely: a constant mixing speed was maintained, simulating blood circulation, the experiment was conducted at the temperature of + 37°C.

### *Statistical processing*

the data obtained were processed by the application package STATISTICA 6.0 (StatSoft, Inc., USA, for the Windows operating system) and Microsoft Excel 2010. The mean value and its standard error (M ± m) was determined. The normal distribution was evaluated by the Shapiro-Wilk criterion. In the normal distribution of the data, the Student's t-test for multiple comparisons was used to compare means. The differences were considered significant at $p < 0.05$. Student's t-parameter was used for normal data distribution of [28].

## RESULTS AND DISCUSSION

These amplitudes of ADP-induced platelet aggregation in a standardized plasma in the control group of animals after 1 h amounted to 41.8 ± 4.8 conventional units (Table 1).

***Table 1 – The effect of Pentoxifylline microparticles based on poly-DL-lactide-co-glycolide and a standard sample of Pentoxifylline when administered per os at the dose of 100 mg/kg per ADP-induced platelet aggregation process***

| Group of animals, conventional units | Observation time | | | | |
|---|---|---|---|---|---|
| | 1 h | 3 h | 5 h | 8 h | 24 h |
| Control | 41.8 ± 4.8 | 47.0 ± 5.1 | 39.4 ± 2.9 | 37.8 ± 2.3 | 42.7 ± 4.8 |
| Pentoxifylline | 23.9±1.9*<br>x=57.2% | 23.8± 1.9*<br>x=50.6% | 40.8 ± 6.8<br>x=103.6% | 42.5 ± 2.8<br>x=112.4% | 40.4±3.9<br>x=95.7% |
| Microparticles of Pentoxifylline on the basis of RLGA | 24.2±1.8*<br>x=57.9% | 27.0±2.2*<br>x=57.4% | 27.9±2.3#*<br>x=70.8% | 27.2±3.2#*<br>x=72% | 27.2±1.7*#<br>x=63.7% |

*Note:*
** – statistically significant (t - Student's criterion) relative to the control group;*
*# – statistically significant (t - Student's criterion) relativeto the Pentoxifylline group*

After a single intragastric administration of Pentoxifylline (at the dose of 100 mg/kg), the degree of ADP-induced platelet aggregation in a standardized plasma after 1 h of administration was 23.9±1.9 conventional units (Table 1), i.e. 42.8% lower than in the control group rats. A similar effect on the platelet aggregation activity after 1 h after the introduction of the observation was also established with the introduction of PLGA-based Pentoxifylline microparticles: the studied parameter was 24.2±4.8 conventional units (Table 1) 42.1% lower than in the experimental group. The high efficacy of Pentoxifylline, as well as microparticles of Pentoxifylline on the basis of PLGA as an antiplatelet agent for ADP-induced platelet aggregation was also recorded 3 hours after the administration (Table 1).

It should be noted that the antiplatelet effect in both experimental groups in the considered time interval was comparable. Thus, in the group with Pentoxifylline, the indicator under study was 23.8±1.9 conventional units (Table 1), and in the group of Pentoxifylline microparticles on the basis of PLGA it was 27.0 ± 2.2 conventional units, while in the control 47.0±5.1 it was conventional units (Table 1). Thus, the reduction in platelet aggregation was 49.4% for the "standard" Pentoxifylline and 42.6% for the PLX-based Pentoxifylline microparticles.

In the study of other time periods (5 h, 8 h and 24 h),

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the process of platelet aggregation established significant differences in the effects on the object under study from the comparator drug.

Thus, in the group of the animals which received Pentoxifylline *per os* at the dose of 100 mg/kg, there were no statistically significant differences from the animals of the control group (Table 1). At the same time, in the animals which received oral PLGA-based microparticles *per os* in a similar dose, significant differences were recorded during the analyzed observation period. So, after 5 hours, the degree of platelet aggregation was 27.9 ± 2.3 c conventional units, after 8 h – 27.2 ± 3.2 conventional units, and after 24 h – 27.2 ± 1.7 conventional units (Tab. 1). In the control, the studied indicator was respectively 39.4 ± 2.9 conventional units, 37.8 ± 2.9 conventional units, and 42.7 ± 4.8 conventional units (Table. 1).

Thus, the prolonged form of Pentoxifylline on the basis of PLGA in the dose of 100 mg/kg taken intragastrically once, unlike the standard Pentoxifylline in a similar dose, has a pronounced antiaggregant activity not only for 1 hour and 3 hours of the experiment, but for 5 hours, 8 hours and 24 hours of the observation, respectively, inhibiting the process of platelet aggregation by 29.2%, 28.04% and 36.3%.

Analyzing the influence of the objects of study on the rate of platelet aggregation, it was established that Pentoxifylline microparticles on the basis of PLGA, unlike Pentoxifylline, significantly inhibit this process during the entire observation period. It was experimentally shown that in the 1st and 3rd hours of the observation, the studied parameter in both experimental groups was statistically significantly lower than in the animals without pharmacological correction (Fig. 1).

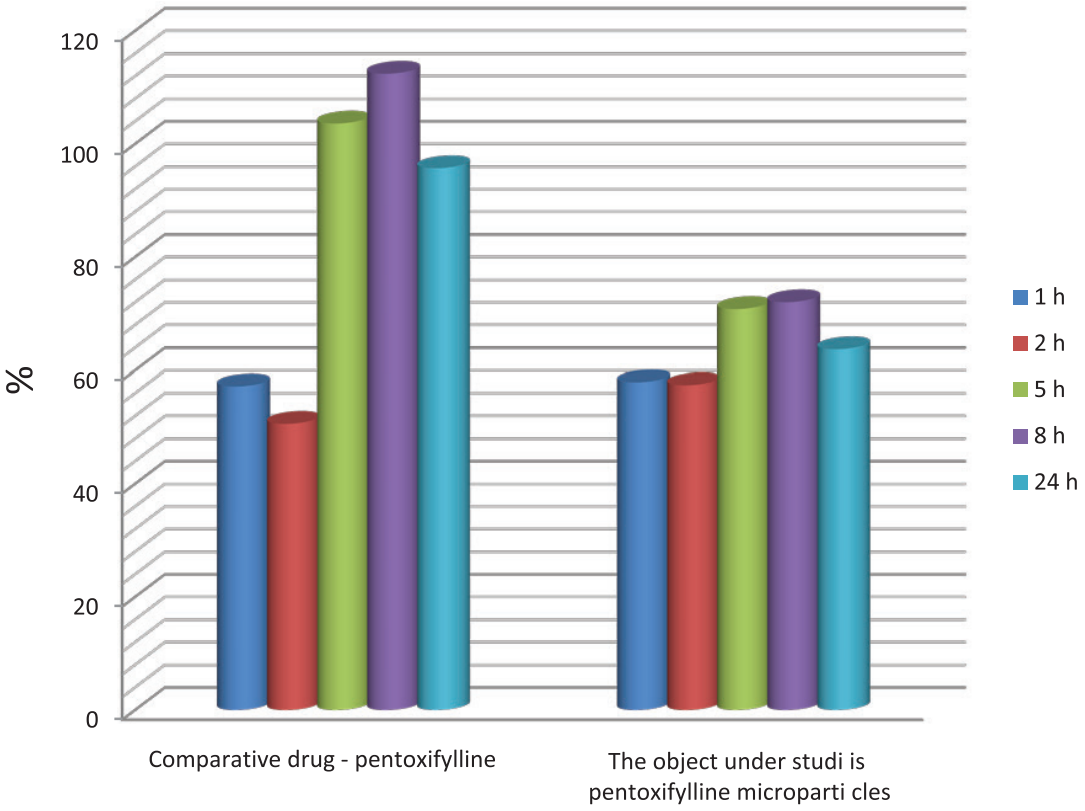


***Figure 1 – The effect of Pentoxifylline and Pentoxifylline microparticles on the basis of PLGA on the rate of ADP-induced platelet aggregation***

Thus, in the control, the rate of platelet aggregation after 1 hour and 3 hours was 52.71 ± 2.12 conventional units and 57.48 ± 1.44 conventional units, respectively. In the group of the animals that received microparticles of Pentoxifylline on the basis of PLGA it was 30.90 ± 1.37 conventional units (after 1 h) and 29.02 ± 1.63 conventional units (after 3 hours), while in the group of the animals receiving the "standard" preparation, the analyzed indicator was 32.20 ± 0.82 conventional units and 33, 62 ± 1.36 conventional units, respectively, after 1 h and 3 h of observation.

Further study of the rate of platelet aggregation indicates a significant difference in the action of Pentoxifylline microparticles based on PLGA and the reference drug, on the dynamics of the process under consideration. In the group of the animals to which the "standard" Pentoxifylline was administered, no statistically significant differences from the control animals on the effects on the platelet aggregation rate at 5, 8 and 24 hours of the experiment were revealed, i.e. the drug's effect was over (Figure 1). At the same time, the use of the innovative

form of Pentoxifylline significantly limits the process of platelet aggregation during the entire observation period (Fig. 1). In addition, it should be noted that the severity of this process in this group of the animals throughout the experiment was comparable.

The generation of $TXA_2$ by platelets and a decrease in the level of cAMP is associated with the fact that ADP is a weak agonist. The recorded effect of PLGA-based Pentoxifylline microparticles on cell aggregability must be associated with the changes in platelet membrane properties.

On the platelet membrane, ADP binds to 3 purinoreceptors (P2Y12, P2X1 and P2Y1). The ionotropic receptor, P2X1, is responsible for the entry of exogenous $Ca^{2+}$ and $Na^{+}$ into the cell; the remaining two P2Y receptors are associated with G-proteins, which carry a stimulation signal inside the cell. In order to develop a complete aggregation when exposed to ADP platelets, a compound of this agonist with both P2Y receptors is required [1]. The fact that under the influence of ADP there is a clear aggregation of platelets, as well as due to the effect of Pentoxifylline, its significant suppression occurs, which proves a significant role of purineregic receptors in the implementation of the pharmacological response to this drug once again.

The comparability of the antiplatelet action of the "standard" Pentoxifylline and its innovative form based on PLGA, which were identified at 1 and 3 hours after oral administration of the objects of study, indicate the preservation of biophase during the implementation of the antiplatelet effect of Pentoxifylline microparticles (Fig. 2).

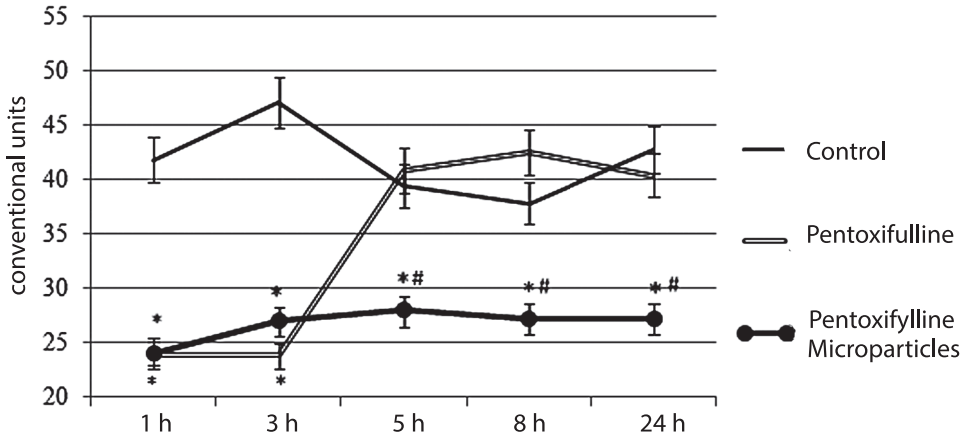


***Figure 2 – The effect of Pentoxifylline and Pentoxifylline microparticles on the basis of PLGA on the process of ADP-induced platelet aggregation***

*Note:*
** – marked statistically significant ($p < 0.05$) shifts of parameters compared with the control;*
*# – marked statistically significant ($p < 0.05$) parameter shifts compared to Pentoxifylline.*

At the same time, the duration of the pharmacological response (24 hours), which was observed in the study of Pentoxifylline microparticles based on PLGA and under the conditions of ADP induction of platelet aggregation, makes it possible to suggest that pharmacodynamic changes are due to the pharmacokinetics of the object of study. The results of the study indicate that PLGA-based Pentoxifylline microparticles effectively reduce (by more than 40%) platelet aggregation in the first 3 hours of the experiment, while the effectiveness of the pharmacological action of ADP induced platelet aggregation is comparable to "standard" Pentoxifylline (Fig. 2).

According to the data obtained during the experiment, Pentoxifylline microparticles based on PLGA (unlike Pentoxifylline) significantly inhibit ADP-induced platelet aggregation within 24 hours.

## CONCLUSION

The use of PLGA-based Pentoxifylline microparticles significantly contributes to prolongation of the action of Pentoxifylline as an antiaggregatory agent for 24 hours.

The results of the experiments showed that PLGA-based Pentoxifylline microparticles are more effective at reducing the ability of platelets to aggregate in the first 3 hours of the study (more than 40%). Besides, it should be noted that the effectiveness of pharmacological action during ADP-induced microparticle platelet aggregation commensurate with the standard sample. According to the data obtained during the experiment, Pentoxifylline microparticles based on poly-DL-lactide-co-glycolide (unlike Pentoxifylline) significantly inhibit ADP-induced platelet aggregation within 24 hours.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# RESULTS OF IMPORT SUBSTITUTION ANALYSIS OF ANTI-CANCER MEDICATIONS IN THE RUSSIAN FEDERATION (2013–2018)

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***The aim.*** *Due to the social importance of anticancer drugs, the aim of the study is to conduct a comparative analysis of the range of domestic and foreign anticancer medications included in the Lists of Vital and Essential Drugs in 2013 and 2018 in the aspect of import substitution.*
***Materials and methods.*** *The study was conducted by comparing the data on the registered anticancer preparations included in the list of Vital and Essential Drugs in the State Register of Medicines in 2013 and 2018. Statistical processing of the data was carried out on the basis of Fisher Z-test method.*
***Results.*** *All in all, there were 286 trade names of anticancer drugs registered in the Russian Federation in 2013. 94 of them, i.e. 33%, were Russian-made. In 2013, there were 19 pharmaceutical substances of domestic production in the analyzed group, which accounted for 27% of 71 INNs (International nonproprietary name). As for 71 INNs, in 2018 there were 393 registered trade names of anticancer drugs. 162 drugs, i.e. 41%, were Russian-made. From 2013 to 2018, a statistically significant positive trend of the increase in the number of domestic anticancer drugs by 8%. was revealed.*
***Conclusion.*** *In order to increase the volume of import substitution in the production of anticancer drugs, it is necessary to provide state support to Russian manufacturers, who register domestic analogues for the first time. A particular attention should be paid to the production of domestic pharmaceutical substances, without which it is impossible to ensure the drug safety of the country.*
***Keywords:*** *drugs, medicines, the State Register of Medicines, import substitution, pharmaceutical substances, anticancer drugs*

# РЕЗУЛЬТАТЫ АНАЛИЗА ИМПОРТОЗАМЕЩЕНИЯ ПРОТИВООПУХОЛЕВЫХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ В РОССИЙСКОЙ ФЕДЕРАЦИИ ЗА 2013–2018 ГОДЫ

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***Цель.*** *В виду социальной значимости противоопухолевых лекарственных препаратов (ЛП) цель исследования – провести сравнительный анализ ассортимента отечественных и зарубежных противоопухолевых ЛП, входящих в перечни жизненно необходимых и важнейших лекарственных препаратов (ЖНВЛП) в 2013 и 2018 гг. в аспекте импортозамещения.*
***Материалы и методы.*** *Исследование проведено сравнением данных о зарегистрированных противоопухолевых препаратах, входящих в перечни ЖНВЛП в 2013 и 2018 гг. в Государственном реестре лекарственных средств. Статистическая обработка полученных данных была проведена методом z-критерия Фишера.*

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***Результаты.*** *Всего в РФ на 2013 год было зарегистрировано 286 торговых наименований противоопухолевых ЛП, из них российского производства 94 препарата, т.е. 33%. Фармацевтических субстанций отечественного производства в анализируемой группе в 2013 году было 19, что составляло 27% от 71 МНН (Международное непатентованное наименование). В 2018 году по 71 МНН зарегистрировано 393 торговых наименования противоопухолевых ЛП, из них российского производства – 162 препарата, т.е. 41%. Выявлена статистически значимая положительная тенденция увеличения количества противоопухолевых препаратов отечественного производства с 2013 года по 2018 – на 8%.*
***Заключение.*** *В целях увеличения объема импортозамещения при производстве противоопухолевых ЛП необходимо обеспечить государственную поддержку российских производителей, впервые регистрирующих отечественные аналоги. Особое внимание следует уделить производству отечественных фармацевтических субстанций, без которых невозможно обеспечить лекарственную безопасность страны.*
***Ключевые слова:*** *лекарственные средства, лекарственные препараты, импортозамещение, фармацевтические субстанции, противоопухолевые лекарственные препараты*

## INTRODUCTION

Ensuring the health of the nation is determined by the state national policy in the field of health care including drug provision of the population.

The organization of the production of domestic drugs is the aim of the state policy in the sphere of providing the population with vital and essential drugs, the production of which is planned to be raised to 90% in the Russian Federation by 2025 [1–4].

Cancer is considered to be one of the important problems of modern health care. It is one of the most common pathologies after insult and myocardial ischemia. And every year the number of cancer patients is growing steadily.

In 2016, 599 thousand cases of malignant neoplasms were detected in the Russian Federation for the first time. Mortality rate due to neoplasms was 201.6 per 100 thousand population. At the end of 2016, more than 3 million patients were registered in territorial oncological institutions [5].

## MATERIALS AND METHODS

Due to the social importance of treating cancer patients, a comparative analysis of the range of domestic and foreign anticancer drugs included in the lists of Vital and Essential Drugs (VED) in 2013 and 2018 in the aspect of import substitution, has been carried out [6, 7]. The list of Vital and Essential Drugs dated 2013, was taken as a starting point due to the fact that in 2013 Strategy of Drug Provision of the Population of the Russian Federation was adopted.

According to the DSM Group data, in 2013, about 90 billion rubles were spent on all anticancer drugs from all sources of financing including individuals, and 95 million packages were purchased [8].

At the end of 2017, about 123 billion rubles were spent on anticancer drugs and 80 million packages were purchased [9]. Thus, there is an increase in the cost value for anticancer drugs – by 4% over 4 years and a decrease in the natural consumption of this group by 15%.

The scientific research was performed sequentially and included three interrelated stages.

The objective of the first stage of the study was to compare the lists of Vital and Essential Drugs adopted in 2013 and 2018. 71 INNs of the drugs coincide in the lists of the VED in 2013 and 2018. The group of anticancer drugs included in the list of VED in 2018 is 65 INNs larger than in the list of VED in 2013.

The task of the second stage of the study was a comparative analysis of the range of the drugs registered in the Russian Federation and the substances for their production, hereby, 71 INNs were included in the both lists (Table 1). In the analysis, the data from the State Register of Medicines were used.

***Table 1. Medicines for treating patients with oncological diseases in Vital and Essential Drugs lists (2013–2018)***

| № | INN | Registered by trade names (assortment positions) | | | | The number of registered Russian substances (assortment positions) | |
|---|---|---|---|---|---|---|---|
| | | Russian manufacturers | | Foreign manufacturers | | | |
| | | 2013 | 2018 | 2013 | 2018 | 2013 | 2018 |
| 1 | Cyclophosphamide | 4 | 4 | 1 | 1 | 1 | 1 |
| 2 | Carmustine | – | – | 2 | 2 | | – |
| 3 | Ifosfamide | 3 | 3 | 1 | 1 | 1 | 1 |
| 4 | Melphalan | – | – | 2 | 2 | – | |
| 5 | Chlorambucil | – | – | 2 | 2 | – | – |
| 6 | Temozolomide | 3 | 9 | 7 | 4 | – | 2 |
| 7 | Methotrexate | 3 | 5 | 3 | 4 | – | – |
| 8 | Pemetrexed | – | 2 | 1 | 1 | – | 1 |
| 9 | Raltitrexed | – | – | 2 | 2 | – | – |

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*Continuation of table 1*

| № | INN | Registered by trade names (assortment positions) | | | | The number of registered Russian substances (assortment positions) | |
|---|---|---|---|---|---|---|---|
| | | Russian manufacturers | | Foreign manufacturers | | | |
| | | 2013 | 2018 | 2013 | 2018 | 2013 | 2018 |
| 10 | Nelarabine | – | – | 2 | 2 | – | – |
| 11 | Gemcitabine | 6 | 5 | 10 | 10 | 1 | 1 |
| 12 | Fludarabine | 1 | 6 | 3 | 8 | 1 | 1 |
| 13 | Capecitabine | 2 | 7 | 4 | 4 | – | 3 |
| 14 | Fluorouracil | 2 | 5 | 4 | 3 | – | – |
| 15 | Cytarabine | 1 | 2 | 3 | 5 | – | – |
| 16 | Vinblastine | 1 | 2 | 2 | 3 | – | – |
| 17 | Vincristines | 1 | 3 | 1 | 2 | – | – |
| 18 | Vinorelbine | 4 | 2 | 4 | 6 | – | – |
| 19 | Etoposide | 1 | 1 | 5 | 5 | – | – |
| 20 | Docetaxel | 4 | 3 | 4 | 6 | – | 1 |
| 21 | Paclitaxel | 3 | 3 | 12 | 12 | – | – |
| 22 | Bevacizumab | – | 1 | 2 | 1 | – | 1 |
| 23 | Trastuzumab | – | 1 | 2 | 1 | – | 1 |
| 24 | Cetuximab | – | – | 1 | 1 | – | – |
| 25 | Gefitinib | – | 2 | 1 | 1 | – | 1 |
| 26 | Dasatinib | – | 1 | 1 | 1 | – | 1 |
| 27 | Imatinib | 5 | 17 | 4 | 10 | 1 | 5 |
| 28 | Sorafenib | – | 1 | 1 | 1 | – | 1 |
| 29 | Sunitinib | – | 1 | 1 | 1 | – | 1 |
| 30 | Asparaginase | 1 | 2 | 1 | 1 | – | – |
| 31 | Bortezomib | 1 | 5 | 1 | 1 | – | 3 |
| 32 | Irinotecan | 3 | 3 | 10 | 10 | – | 1 |
| 33 | Medroxyprogesterone | 1 | 1 | 5 | 5 | – | – |
| 34 | Leuprorelin | – | – | 3 | 3 | – | – |
| 35 | Triptorelin | – | 1 | 1 | 2 | – | 1 |
| 36 | Tamoxifen. | 4 | 4 | 7 | 7 | – | – |
| 37 | Fulvestrant | – | – | 1 | 1 | – | – |
| 38 | Bicalutamide | 7 | 8 | 7 | 6 | – | 3 |
| 39 | Flutamide | 3 | 3 | 4 | 8 | – | – |
| 40 | Anastrozole | 3 | 5 | 7 | 6 | – | 1 |
| 41 | Filgrastim | 6 | 7 | 7 | 8 | – | 4 |
| 42 | Interferongamma | 1 | 1 | - | - | 1 | 1 |
| 43 | Anoxemiabromide | 1 | 1 | - | - | 1 | 1 |
| 44 | Vaccine for treatment of bladder cancer BCG | 1 | 1 | - | - | 1 | 1 |
| 45 | Glatirameracetate | 1 | 3 | 1 | 1 | – | 1 |
| 46 | Glutamyl-cysteinyl-glycinedisodium | 1 | 1 | - | - | 1 | 1 |
| 47 | Megluminacridonacetate | 1 | 1 | - | - | 1 | 1 |
| 48 | Tiloron | 4 | 7 | 3 | 3 | 4 | 4 |
| 49 | Abatacept | – | – | 1 | 1 | – | – |
| 50 | Mycophenolatemofetil | 2 | 2 | 4 | 6 | – | 2 |
| 51 | Mycophenolicacid | – | – | 1 | 1 | – | – |
| 52 | Everolimus | – | – | 1 | 1 | – | – |
| 53 | Infliximab | 1 | 1 | 3 | 3 | – | – |
| 54 | Etanercept | – | – | 1 | 1 | – | – |
| 55 | Basiliximab | – | – | 1 | 1 | – | – |
| 56 | Tocilizumab | – | – | 1 | 1 | – | – |
| 57 | Tacrolimus. | – | 6 | 2 | 12 | – | – |
| 58 | Cyclosporine | – | – | 6 | 6 | – | – |

*Continuation of table 1*

| № | INN | Registered by trade names (assortment positions) | | | | The number of registered Russian substances (assortment positions) | |
|---|---|---|---|---|---|---|---|
| | | Russian manufacturers | | Foreign manufacturers | | | |
| | | 2013 | 2018 | 2013 | 2018 | 2013 | 2018 |
| 59 | Azathioprine | 1 | 1 | 1 | 1 | 1 | 1 |
| 60 | Lenalidomide | – | 2 | 1 | 2 | – | 2 |
| 61 | Interferon alpha-2b | 5 | 5 | 3 | 3 | 4 | 4 |
| 62 | Busulfan | – | – | 2 | 2 | – | – |
| 63 | Hydroxycarbamide | – | 3 | 3 | 6 | – | 1 |
| 64 | Goserelin | – | – | 1 | 1 | – | – |
| 65 | Ibandronicacid | – | – | 2 | 2 | – | – |
| 66 | Interferon alpha-2a | – | – | 1 | 1 | – | – |
| 67 | Lomustine | – | – | 1 | 1 | – | – |
| 68 | Mercaptopurine | – | 1 | 3 | 3 | – | 1 |
| 69 | Nilotinib | – | – | 1 | 2 | – | 1 |
| 70 | Rituximab | 1 | 1 | 1 | 1 | – | 1 |
| 71 | Tretinoin | – | – | 3 | 3 | – | – |

## RESULTS AND DISCUSSION

In total, in the Russian Federation 286 trade names of anticancer drugs were registered in 2013. 94 of them, i.e. 33%, were Russian-made. In 2013, in the analyzed group there were 19 pharmaceutical substances of domestic production which accounted for 27% of 71 INNs.

In 2018, 71 INNs were registered under 393 trade names of anticancer drugs, 162 of which, i.e. 41%, were Russian-made. Thus, there is an 8% increase in the share of domestic drugs in the anticancer group.

In 2018, 37 pharmaceutical substances of domestic production were registered in the analyzed group. That accounted for 51% of the 71 INNs.

Compared with 2013, 11 INNs of Russian production appeared additionally in 2018.

To confirm the reliability of the results obtained, statistical processing on the basis of the Fisher z-test method was performed.

The use of the program for comparing the indicators of the two groups on the Z-criterion, was the most acceptable for the purposes of our study. The Z-criterion is one of non-parametric criteria. The data of $Z = 4.54$ ($p = 0.0001$) shows statistically significant differences in the group of anticancer drugs included in the List of Vital and Essential Drugs made in Russia in 2013 and 2018.

As for 33 INNs, the number of domestic and foreign manufacturers of medicines have not changed.

In 2018, pharmaceutical substances were produced by 37 domestic enterprises, i.e. 51% more than in 2013. But at the same time a certain tendency was revealed. It was connected with the fact that the same medications began to be produced by several manufacturers simultaneously. Thus, there were 43 trade names, i.e. about 26% out of 162 registered domestically manufactured medications which referred to 5 MNNs (Imatinib, Temozolomide, Tacrolimus, Fludarabine and Bortezomib). The question arises why domestic manufacturers have chosen those INNs and how the number of the registered drugs is related to the real need for anticancer drugs.

The approaches to the registration of the 5 domestic and foreign drugs given above in the lists of INN, are not clear. It would be logical if an increase in the number of the registered drugs of domestic production should be accompanied by a decrease in the number of imported anticancer drugs registered in the Russian Federation.

However, this ratio is observed only for Temozolomide and Bortezomib. In 2013–2018 the number of Russian manufacturers of Temozolomide increased from 3 to 9, and the number of foreign manufacturers decreased from 7 to 4. From 2013 to 2018, the number of Russian manufacturers of Bortezomib increased from 1 to 5, but the number of foreign manufacturers has not changed.

As for the remaining 3 medications, there is a tendency of a parallel increase in the number of registered domestic and foreign drugs.

In particular: from 2013 to 2018, the number of Russian manufacturers of Imatinib increased from 5 to 17, and the number of medications of foreign manufacturers increased from 4 to 10. During this period, the number of Russian manufacturers of Fludarabinaz increased from 1 to 6, and the number of drugs of foreign manufacturers increased from 3 to 8.

The situation with Tacrolimus is similar: in 2018 6 Russian manufacturers were registered (in 2013 there was none), and the number of foreign manufacturers increased by 8 (from 2 to 10).

At the third stage, 65 INNs have been analyzed. They make up a group of anticancer drugs, first included in the list of VED in 2018 (Table 2).

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*Table 2. Medicines for treating patients with oncological diseases in Vital and Essential Drugs lists (2018)*

| № | INN | Registered by trade names (assortment positions) | | The number of Registered Russian substances (assortment positions) |
|---|---|---|---|---|
| | | Russian manufacturers | Foreign manufacturers | |
| 1 | Bendamustine | – | 1 | – |
| 2 | Dacarbazine | 2 | 2 | – |
| 3 | Azacitidine | – | 1 | – |
| 4 | Daunorubicin | 3 | – | 2 |
| 5 | Doxorubicin | 7 | 4 | 1 |
| 6 | Idarubicin | 3 | 1 | – |
| 7 | Mitoxantrone | 4 | 2 | – |
| 8 | Epirubicin | 2 | 2 | – |
| 9 | Bleomycin | 4 | 2 | 1 |
| 10 | Mitomycin | 1 | 2 | – |
| 11 | Carboplatin | 3 | 6 | 2 |
| 12 | Oxaliplatin | 4 | 9 | 1 |
| 13 | Cisplatin | 4 | 3 | – |
| 14 | Procarbazine | – | 1 | – |
| 15 | Brentuximabvedotin | – | 1 | – |
| 16 | Nivolumab | – | 1 | – |
| 17 | Obinutuzumab | – | 1 | – |
| 18 | Panitumumab | – | 1 | – |
| 19 | Pembrolizumab | 1 | 1 | – |
| 20 | Pertuzumab | – | 1 | – |
| 21 | TrastuzumabEltanin | – | 1 | – |
| 22 | Afatinib | – | 1 | – |
| 23 | Vandetanib | – | 2 | – |
| 24 | Dabrafenib | – | 2 | – |
| 25 | Ibrutinib | 1 | 1 | – |
| 26 | Krizotinib | – | 1 | – |
| 27 | Nintedanib | – | 1 | – |
| 28 | Pazopanib | – | 2 | – |
| 29 | Regorafenib | – | 1 | – |
| 30 | Rukolaine | – | 1 | – |
| 31 | Trametinib | – | 2 | – |
| 32 | Erlotinib | – | 3 | – |
| 33 | Aflibercept | – | 2 | – |
| 34 | Wiimotelib | – | 1 | – |
| 35 | Carfilzomib | – | 1 | – |
| 36 | Tumor necrosis factor alpha-1 (thymosin recombinant) | 1 | 1 | 1 |
| 37 | Eribulin. | – | 1 | – |
| 38 | Buserelin | 2 | – | 2 |
| 39 | Goserelin | – | 1 | – |
| 40 | Insulated | – | 1 | – |
| 41 | Abiraterone | 3 | 2 | 3 |
| 42 | Degarelix | – | 1 | – |
| 43 | Interferon beta-1a | 1 | 2 | 1 |
| 44 | Interferon beta-1b | 2 | 1 | 1 |
| 45 | Peginterferon alpha-2a | – | 1 | – |
| 46 | Peginterferon alpha-2b | 2 | 1 | 1 |
| 47 | Interferon beta-1a | – | 1 | – |
| 48 | Cepeginterferon alpha-2b | 1 | 1 | 1 |
| 49 | Alemtuzumab | – | 2 | – |
| 50 | Apremilast | – | 1 | – |

*Continuation of table 1*

| № | INN | Registered by trade names (assortment positions) | | The number of Registered Russian substances (assortment positions) |
|---|---|---|---|---|
| | | Russian manufacturers | Foreign manufacturers | |
| 51 | Vedolizumab | – | 1 | – |
| 52 | Leflunomide | 4 | 2 | – |
| 53 | Natalizumab | – | 1 | – |
| 54 | Teriflunomide | 1 | 1 | 1 |
| 55 | Tofacitinib | – | 1 | – |
| 56 | Fingolimod | – | 1 | – |
| 57 | Eculizumab | – | 1 | – |
| 58 | Adalimumab | – | 1 | – |
| 59 | Golimumab | – | 1 | – |
| 60 | Certolizumabpegol | – | 1 | – |
| 61 | Canakinumab | – | 1 | – |
| 62 | Secukinumab | – | 1 | – |
| 63 | Tocilizumab | – | 1 | – |
| 64 | Ustekinumab | – | 1 | – |
| 65 | Pirfenidone | – | 1 | – |

As for 65 INNs, 153 trade names included in this group of drugs were registered in 2018. 56 of them, i.e. 35%, were made in Russia.

The especially alarming factor is that out of 65 INNs included in the list of VED in 2018, only 13 substances (20%) and 22 INNs are produced in Russia (34%), and 38 registered INNs are produced by only one foreign manufacturer.

Thus, the conducted study has shown that out of 136 INNs of anticancer drugs, which were included in the list of Vital and Essential Drugs in 2018, 546 trade names were registered: 218 drugs are Russian-made, which is 40% of all the registered anticancer drugs in the Russian Federation.

## CONCLUSIONS

The study has shown a statistically significant positive though not high enough tendency to increase the number of anticancer drugs of domestic production from 2013 to 2018 – by 8%.

In order to optimize the import substitution of anticancer drugs, it is necessary to do the following.

1. Determine the real need of health care in the Russian Federation for the entire range of anticancer drugs.

2. Taking into account a high social significance of this group of medications, the possibility of forming a state order for their production and distributing it among domestic producers, providing them with state support, should be considered.

3. Limit the number of registered generics of anticancer drugs due to a more thorough assessment of their benefits.

4. Particular attention should be paid to the production of domestic pharmaceutical substances, without which it is impossible to ensure the drug safety of the country.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# TO THE 100TH ANNIVERSARY OF PROFESSOR O.K. KOZMINYKH

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*In connection with the 100th anniversary of Professor Oleg Kozmich Kozminykh, the brief biographical data are presented herein. O.K. Kozminykh made a great contribution to the development of pharmaceutical education and science in the Urals being the leader of Perm Pharmaceutical Institute for more than 20 years. Based on documents and personal memories, the biographical essay is provided and general data of life as well as scientific, pedagogical and public activities of O.K. Kozminykh are summarized here.*
***Keywords:*** *Kozminykh, biography, pharmaceutical education and science, Perm Pharmaceutical Institute*

# К 100-ЛЕТИЮ СО ДНЯ РОЖДЕНИЯ ПРОФЕССОРА О.К. КОЗЬМИНЫХ

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*В связи с юбилеем – 100-летием со дня рождения – приведены краткие биографические сведения о профессоре Козьминых Олеге Козьмиче, который внёс большой вклад в развитие фармацевтического образования и науки на Урале и более 20 лет возглавлял Пермский фармацевтический институт. На основании документов и личных воспоминаний представлен биографический очерк и перечислены основные даты жизненного пути, научной, педагогической и общественной деятельности О.К. Козьминых.*
***Ключевые слова:*** *Козьминых, биография, фармацевтическое образование и наука, Пермский фармацевтический институт*

In April 2019 the 100th anniversary of the outstanding scientist and teacher Oleg Kozmich Kozminykh (Fig. 1) is celebrated. He was a front-line soldier, a knight of Order of the Red Star, the Second World Patriotic War of the 2nd Degree, Order of the Red Banner of Labor. He devoted a significant part of his work to the development and improvement of pharmaceutical education and science in the Urals, working as the rector of Perm State Pharmaceutical Institute for two decades.

O.K. Kozminykh was born in Perm on April 16th, 1919 in the family of an employee. In 1940 he graduated from the Perm Pharmaceutical Institute with honors, and worked at the Pharmacology Department of Perm Medical Institute under the guidance of Professor A.K. Sangailo up to the pre-war conscription [1].

From November 13th, 1940 to September 26th, 1953 Oleg Kozmich Kozminykh served in the Red Army of the USSR. He participated in the Great Patriotic War of 1941–1945. As an officer of the medical service, he served as Chief of pharmacy rifle regiment, Chief of medical sup-

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ply infantry division, Head of the army and front medical warehouses of the 2nd and 3rd Ukrainian fronts. O.K. Kozminykh served in the Armed Forces of Far Eastern military district (248th Separate cadet rifle brigade, 42nd rifle division of the 87th rifle regiment, 233rd rifle regiment of the separate Red Banner rifle division). He fought in the Armed Forces of Stalingrad, Don, Steppe fronts (24th and 65th Army, 233rd rifle regiment of the separate Red Banner Kremenchug-Znamenskaya rifle division; 284th mechanized brigade). He served in the southern group of troops, as well as in the Carpathian and the Urals military districts. Before the Stalingrad battle, until February 1943, he fought in the 233rd Red Banner Kremenchug-Znamensky rifle division. O.K. Kozminykh was fighting his way from Stalingrad to Vienna, he was a participant of the Stalingrad battle. At the front in 1943 he joined the Communist Party of the USSR. From 1944 to 1950 he served in military units in Bulgaria and Romania. As a result of severe front-line contusion, as well as after the disease of malaria, received in the Danube swamps of Romania, O.K. Kozminykh was treated several times in hospitals of Constanta, Bucharest, Odessa, Kiev and Proskurov.

For his service in the Armed Forces of the USSR during the Great Patriotic War and post-war labor merits Oleg Kozmich Kozminykh was awarded Order of the Red Star, World War 2, and Order of the Red Banner of Labor, as well as 13 medals of the USSR, including the following: "For Military Merit" for the defense of Stalingrad, for the capture of Budapest, Vienna, the liberation of Belgrade, "For the Victory over Germany in the Great Patriotic War of 1941–1945", "20 years of Victory in the Great Patriotic War", "30 years of Victory in the Great Patriotic War", "40 years of Victory in the Great Patriotic War", "30 years of the Soviet Army and Navy", "50 years of the Armed Forces of the USSR", "60 years of the Armed Forces of the USSR", "Victory in the battle of Stalingrad 1943–2003 (60 years)", as well as commemorative signs of a veteran of the Steppe and 2nd Ukrainian fronts, a veteran of the 53rd Army, a soldier of 1941–1945.

Here are a few lines from the records of personal memories: "At the end of the war, six years after joining the army in the autumn of 1946, I have got my first vacation and came home to Perm. Entering the Department of Pharmacology of the Medical Institute, a place of previous work, I was presented to the staff Head of the Department Professor A.K. Sangailo. I met a young assistant Lobantseva Emilia Mikhailovna. We became friends, visited theaters, movies, and soon the decision came to get married. All employees of the Department celebrated the wedding-party. Four days later we went abroad to the place of service. There I went about my daily life of a soldier: garrisons, moving from country to country. Our daughter was born in Romania. Finally in 1953 we insisted on transferring to Perm. Retired, I entered a PhD programme. All my further life got connected to pedagogical and scientific work...".

Scientific and pedagogical activities of O.K. Kozminykh began after the war. Having retired from active duty, from 1954 to 1957, he studied the post doctorate (aspirant) course at the Department of Chemistry in Perm Pedagogical Institute the n. a. Molotov, where as his Chief Professor Nikolai Semenovich Kozlov, he taught and performed scientific work at that time. N.S. Kozlov worked in Perm in 1946–1967. He was elected an academician of the Academy of Sciences of the Belarusian SSR in 1966, and in early 1967 he went to Minsk, heading the Institute of Physical Organic Chemistry of the BSSR Academy of Sciences [2, 3].

***Figure 1 – O.K. Kozminykh (1985)***

The main topic of research of Oleg Kozmich was the chemistry of quinoline and benzoquinoline derivatives. In December 1957, at the end of the post-graduate course, O.K. Kozminykh defended his thesis in the "organic chemistry" on the topic: "Catalytic synthesis of nitro-, amino- and sulfamido derivatives of 2-phenylquinoline and 2-phenyl-5,6-benzoquinoline" with the award of the degree of Candidate of Chemical Sciences. The supervisor of his thesis was Professor Kozlov [4].

In the period from 1957 to 1958 O.K. Kozminykh headed the research group of organic intermediates and dyes of the Central Laboratory of the Perm chemical factory n. a. S. Ordzhonikidze. He also worked as an assistant and associate professor at the Perm Agricultural and Pedagogical Institutes. In the late 50-s O.K. Kozminykh

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studied some production aspects of the chemistry of organic aniline dyes, polymers and improved technological methods for obtaining a number of reagents (nitrobenzene, aniline).

From October 1958 to July 1959 Oleg Kozmich was a lecturer at the Chemistry Department of the Perm State Agricultural Institute. In the period from July 1959 to November 1962 O.K. Kozminykh worked as an associate professor of the Chemistry Department of the Perm State Pedagogical Institute. The founder of the scientific school of organic chemistry and the Head of research of the Department of Chemistry of the Pedagogical Institute was Professor N.S. Kozlov, who was the Head of the Department until 1967. Since late 50's N.S. Kozlov proposed a new direction: the study of catalytic condensation reactions of azomethines with *CH*-acids in the synthesis of nitrogen derivatives of linear and *N*-heterocyclic compounds. The development of this topic was started by the staff of the Department of Chemistry of the Perm Pedagogical Institute. N.S. Kozlov and his students – Z.A. Abramova, E.A. Britan, N.D. Zueva, B.I. Kiselev, O.K. Kozminykh, A.D. Nikolayev, V.D. Pak, S.Ya. Chumakov, I.A. Shur, etc. – found out that under the conditions of catalytic protonation a nucleophilic addition of CH-acids by azomethine bond of Schiff bases occurs there. As a result, various amino ketones, amino acid derivatives, substituted quinolines and their analogues were obtained [5, 6].

In November 1962 O.K. Kozminykh was appointed the rector of Perm State Pharmaceutical Institute and worked in this position for 21 years. In 1973 he was approved as a professor. Oleg Kozmich devoted a lot of efforts and energy to the development and improvement of the Perm Pharmaceutical Institute. This small institute eventually became one of the largest pharmaceutical educational institutions in the country, the staff of teachers reached 260 people, at full-time, correspondence faculties and the faculty of specialization and improvement of pharmacists trained about 3900 students and listeners there. Two academic buildings and two dormitories were built. The training and production base was replenished with a nursery of medicinal plants, the student sports camp was created. Training of scientific and pedagogical personnel has improved, scientific production of the Institute increased [7, 8].

For merits in development of Pharmaceutical Institute and training of personnel O.K. Kozminykh was awarded Order of the Labor Red Banner, medals "For valiant work" and "Veteran of labour ", memorable signs "For excellent progress in work of the Higher School", "Excellent health care organizer" and other awards. For many years of fruitful scientific and pedagogical activity and in connection with the 60th anniversary of his birth O.K. Kozminykh was awarded a diploma of the Presidium of the Supreme Soviet of the RSFSR. Oleg Kozmich was also repeatedly awarded by diplomas of the Perm Regional Committee of the CPSU and the Regional Executive Committee, the Regional Committee of the Komsomol, the city Committee of the CPSU, the Central and regional committees of the Trade Union of medical workers, the Central and regional board of the society "Knowledge" of the RSFSR, the Russian Republican Council of the "Burevestnik" community. O.K. Kozminykh made presentations at scientific and methodological symposia, participated in the all-Union and national conferences and meetings as well. From 1983 to 1987 O.K. Kozminykh worked as a professor at the Department of Inorganic Chemistry at the Perm Pharmaceutical Institute.

For years at the Perm Pharmaceutical Institute Oleg Kozmich Kozminykh successfully carried out research work in the field of catalytic synthesis of quinoline and benzoquinoline derivatives, he studied the properties of liquid-phase catalysts based on vanadium and molybdenum oxides, supervised graduate students, advised applicants on candidate dissertations, participated in the preparation of candidates of sciences of sciences, advised applicants on PhD theses, participated in the training of Candidates of Sciences [5, 9, 10–12]. O.K. Kozminykh is the author of more than 70 scientific, educational and popular scientific works, including six author's certificates for inventions of the USSR. The inventions, which are authored by O.K. Kozminykh, are related to new technologically significant methods of obtaining of formaldehyde, acetaldehyde, and devoted to the results of the development of methods for preventing steel flooding, the creation of catalysts for oxidation reactions for chemical and technological purposes [11, 12].

A detailed review of the scientific work of Oleg Kozmich Kozminykh in May 1972 gave academician N.S. Kozlov, who had become the Director of the Research Institute of Physical Organic Chemistry of the Academy of Sciences of the Belarusian SSR by that time. He, in particular, wrote: "...the theme of a number of works by O.K. Kozminykh is the study of the conditions and mechanism of reaction of catalytic condensation of acetylene with Schiff's bases, obtaining reaction products as new compounds of a number of 2-phenylquinoline and 2-phenyl-5,6-benzoquinoline series, and the study of their properties."

According to the memoirs of professor Vladimir Georgievich Belikov (worked as the rector of the Pyatigorsk Pharmaceutical Institute, later named as Pyatigorsk Pharmaceutical Academy in 1965–1996; the article [13] is devoted to his memory): "...Kozminykh Oleg Kozmich, the rector of the Perm Pharmaceutical Institute, participated in the Great Patriotic War for many years, graduated from the same Institute. There he took post-graduate courses, defended his thesis, worked in various teaching positions, and from 1962 to 1983 was the rector. Despite being very busy with administrative activities, he was successfully engaged in research work on the study of physical and chemical properties of liquid-phase catalysts. In 1973 he was awarded a scientific

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title of Professor. Oleg Kozmich was actively engaged in social activities. He was awarded Order of the Red Star and Order of the Red Banner of Labor, five medals, and a number of other awards of the Ministry of Health of the RSFSR. Many times I have been in Perm at the meetings and as a reviewer, visited the Kozminykhs, their family house. Oleg Kozmich was very friendly to me. He is a true Permian, who loves his small country homeland. He showed me a lot of historical places. I was particularly impressed by the Kungur cave, located about 100 km from Perm, with underwater lakes and various labyrinths. Many other interesting places in Perm have been also shown to us, and wherever we have been, including the house of teachers, we were greeted by true hospitality, friendliness, goodwill. Oleg Kozmich is a very kind person, and none of my requests to him (and also to me) remained unanswered. Reviews of articles and dissertations, all these in the most benevolent form, not excluding critical remarks, were performed in the shortest possible time ..." [14].

Since the end of the 70-s O.K. Kozminykh devoted considerable time to writing and publishing educational and methodical collections and manuals for the study of the inorganic chemistry course for students of the Perm Pharmaceutical Institute. By 1986, 13 methodological elaborations comprising more than 600 pages had been prepared and published.

Oleg Kozmich carried out active public work. He was elected the Deputy of the Perm City Council of People's Deputies (1973–1977) more than once, for twenty years he was a member of the Leninsky District Committee of the Communist party of Perm (1963–1983). O.K. Kozminykh took an active part in the work of the regional Committee of Trade Unions of Medical Workers (he was a member of this regional Committee in 1964). He was a member of the editorial Board of the Journal "Pharmacy", the Central Office of the all-Union Scientific Society of Pharmacists of the USSR, a member of the Methodical Commission on Higher Education of the Ministry of Health of the USSR.

In 1987 O.K. Kozminykh got retired. There are personal memories of Oleg Kozmich about the pedigree of his family since 1794 (he kept records since the pre-war years), which are collected by us for a separate edition. After a serious and long illness on March 29th, 2003 at the age of 83, Oleg Kozmich passed away. The bright memory of Oleg Kozmich Kozminykh has been preserved by many former students, teachers and employees, military and civilian people, relatives and friends. Even during his lifetime he was devoted to separate sections in books [1, 2] and articles [15, 16]. In memory of Oleg Kozmich Kozminykh, an obituary was published in the "Chemical and Pharmaceutical Journal" [17]. The details about this also appeared in his works [18, 19]. The full list of publications by O.K. Kozminykh was presented earlier in the memorable article [20].

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