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Investigation Of the Composition of The Veterinary Preparation Trametin Plus

V.A. Chkhenkeli[2]*, G.D. Chkhenkeli[1], A.A. Nikonova[3], A.G. Gorshkov[3]

Biotechvet LLC

[1]105A/12, Dekabrskikh Sobytii St., Irkutsk, 664007, Russian Federation

Irkutsk State University

[2]Lenina St, Irkutsk, 664003, Russian Federation

Limnological Institute SB RAS

[3]Ulan – Batorskaya St., Irkutsk, 664033

Corresponding Author: V.A. Chkhenkeli, 105A/12, Dekabrskikh Sobytii St., Irkutsk, 664007, Russian Federation

Irkutsk State University

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Abstract

For the prevention and treatment of associated gastrointestinal and respiratory diseases in young farm animals, a new veterinary drug Trametin Plus is proposed, obtained on the basis of xylographic fungi using biotechnology methods. The properties of biological products obtained on the basis of microorganisms and fungi depend on the biologically active substances that make up their composition. In the literature, there is scattered information about the features of lipogenesis and the composition of fatty acids synthesized by wood-destroying fungi. The paper presents data on the study of the qualitative and quantitative composition of fatty acids, a qualitative analysis of volatile polar and non-polar organic compounds of the drug. It has been established that the total concentration of fatty acids is 70 μg/g of the preparation. Of these, 50.0% are free fatty acids, the esterified acids of which account for 50.0% of the total mass of fatty acids. Among the dominant non-polar volatile components of Trametin Plus, squalene can be noted. Organic aliphatic amino acids such as glycine, arginine, and β-alanine with a small molecular weight are presented as minor non-polar volatile components of Trametin plus. The analysis data confirm the multicomponent nature of the drug, which determines its various biological properties, namely, antibacterial, antiviral, antioxidant and immunostimulant activity, which determines the high therapeutic and prophylactic efficacy of the veterinary drug Trametin Plus.

Keywords: *xylographs, veterinary drug, fatty acids, methyl esters, chromato-mass spectrometry*

Introduction

Improving the system of measures to reduce the sickness rate of young farm animals includes the use of new therapeutic and prophylactic drugs. As such a preparation, the new Trametin Plus is proposed, obtained on the basis of the culture liquid during liquid-phase cultivation of the producer Trametes pubescent (Shumach. Fr.) Pilat. strain 0663 from the Collection of BIN RAS L.L. Komarov [1-5].

In the literature, there is information about the features of lipogenesis and the composition of lipids of lower fungi, and algae, while for higher fungi, in particular, for basidiomycetes, similar data are rather scattered. The composition of free fatty acids of the genus Basidiomycetes is poorly studied. Research is limited to single works on the study of lipids of mycothallus [6-16]. However, it is well known that fatty acids have pharmacological activity in relation to various nosologies in both humans and animals. The aim of this work was to study the qualitative and quantitative composition of fatty acids, and qualitative analysis of volatile polar and non-polar organic compounds of the veterinary drug Trametin Plus.

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EXPERIMENTAL PART

The determination of fatty acids was carried out according to the method of A.A. Nikonova et al. [17].

Extraction of lipids from Trametin Plus

Lipids were extracted from parallel weighed portions with a Folch mixture (chloroform–methanol, 2: 1, by volume) in plastic Eppendorf tubes with a capacity of 2 ml, shaking and keeping them in an ultrasonic bath (1.2 ml × 3 × 5 min). The extracts were combined in glass tubes for centrifugation, 1.2 ml of water was added, emulsified, and centrifuged at 3000 rpm. Prior to the extraction of lipids from sponges, 100 µL of 8% H_2SO_4 in water was added to weighed portions (wet weighing 0.02 g at 97% humidity), and after the color changed from green to yellow, lipids were extracted after 2 min. Extraction of lipids from sponges was carried out 1 time, 350 µl of water was added to the extract.

Acid esterification of fatty acids and production of methyl esters of fatty acids (total content) of Trametin Plus

The chloroform layer of the extract (bottom) was transferred into glass penicillin flasks with a capacity of 10 ml, evaporated to dryness in a flow of argon, 4.5 ml of 2% H_2SO_4 in methanol was immediately added, tightly covered with foil, and placed in a thermostat at 55°C for 1.5 h. After methanolysis fatty acid solutions were cooled to room temperature, 0.8 ml of n-hexane was added. FAMEs were extracted from the resulting solutions with n-hexane (3 mL × 2 × 2 min). Before the second extraction, 1 ml of water was added to the solutions. The extracts were concentrated to 1 ml in an argon flow and dried over anhydrous Na_2SO_4. The extracts were analyzed by GC-MS.

Separate production of methyl esters of esterified and free fatty acids under conditions of changing the pH of Trametin Plus

To weigh portions of isolated lipids (0.2 g wet weight) were added 2 ml of 0.4 M NaOH solution in methanol, and placed in an ultrasonic bath for 5 min. The resulting MEs of esterified FAs were extracted with n-hexane (3 ml × 2), washed with water, dried over anhydrous Na_2SO_4, and concentrated in an argon flow to a volume of 1 ml. Extracts of ME FEA were analyzed by GC. To the remaining alkaline solution 3 ml of water and 0.15 g of copper (II) sulfate ground in a porcelain mortar was added, and then shaken for 5–10 min until the blue color of Cu_2SO_4 turned green, $Cu(OH)_2$ (up to pH 6.0), without overkeeping the solution to avoid the transition of $Cu(OH)_2$ to black CuO, which can oxidize FA. Free FAs were extracted with n-hexane (3 ml × 2). Hexane was evaporated and the acid esterification of FFA was carried out. The extracts were analyzed by GC-MS.

Analysis of fatty acid methyl esters (qualitative) by gas chromatography with mass spectrometric detection of extracts

The extracts were analyzed on a 6890B GC System, 7000C GC/MS Triple Quad chromato-mass spectrometer (Agilent, United States) with an Optima-17 column (30 m × 0.25 mm, 0.25 µm) from Macherey-Nagel (Germany). Injector temperature 280 °C; quadrupole temperature 150 °C; ion source temperature 230 °C; type of ionization - electron impact; ionization energy 70 eV; sample volume 2 µl in splitless mode; temperature of the GC-MSD interface unit 310 °C; column flow 2.54 ml/min. Chromatography was carried out under conditions of heating the column from 80°C (hold 0.5 min) to 290°C at a rate of 2°C/min and then heating to 310°C (hold 5 min) at a rate of 5°C/min. Total analysis time 115 min. Peaks were detected in the m/z range of 40–500. Mass spectra were identified using the NIST Mass Spectral Search Program for the NIST Mass Spectral Library (V. 2.2) software.

Quantitative determination of FAME fatty acids by GC-MS

Quantitative determination of FAMEs was carried out by the internal standard method, for which 50 µL of a standard solution of dodecyl ether ($C_{20}H_{42}O$) in n-hexane (1 mg/mL) was added to the extracts before analysis. The detector was calibrated in the ΣFAME range from 40 to 540 µg per sample using standards (Supelco, USA) “35 F.A.M.E. Mix, C4–C24, 100 mg neat” and “Methyl cis-4,7,10,13,16,19-Docosahexaenoic ester, 10 mg/mL in heptane” (70–1000 µg per sample). Calibration factors have been determined for individual acids and for groups of acids (saturated, monounsaturated, polyunsaturated).

Extraction of non-polar organic components of Trametin Plus

Extraction of non-polar components of the sample was carried out from 1.0 g weighed portions of Trametin. The portion of the preparation was placed in a glass vial and extracted with 3 ml of n-hexane three times (1 min). The extracts were combined, washed with distilled water, dried over anhydrous sodium sulfate, concentrated in an argon flow to a volume of 0.5 ml, and analyzed by GC-MS in the full scan mode of the mass spectrum using the NIST Library of Mass Spectra software and the Mass Spectral Search Program for the NIST Mass Spectral Library (V. 2.2).

Extraction of polar organic components of

Trametin Plus

sample was carried out from weighed portions of Trametin Plus weighing 1.0 g. ml), methanol (5 ml) and distilled water (10 ml) under vacuum. The elution of the sample concentrated on the cartridge was carried out with ethanol (5 ml) under vacuum. The extract was concentrated in a flow of argon to a volume of 0.5 ml and analyzed by GC-MS in full mass spectrum scanning mode using the NIST Mass Spectral Library software and Mass Spectral Search Program for the NIST Mass Spectral Library (V. 2.2).

Method 2. Extraction of the polar components of the sample was carried out from weighed portions of Trametin Plus weighing 1.0 g. Hydrochloric acid HCl was added to the weighed portion of the preparation to pH 2.0. Extraction was carried out with 3 ml of methylene chloride three times for 1 min. The extracts were combined, washed with distilled water, dried over anhydrous sodium sulfate, concentrated in an argon flow to a volume of 0.5 ml, and analyzed by GC-MS in the full scan mode of the mass spectrum using the NIST Library of Mass Spectra software and the Mass Spectral Search Program for the NIST Mass Spectral Library (V. 2.2).

Method 1. Extraction of the polar components of the

Spectral Library (V. 2.2).

Qualitative analysis of polar and non-polar components of samples by gas chromatography with mass spectrometric detection

The extracts were analyzed on a 6890B GC System, 7000C GC/MS Triple Quad chromato-mass spectrometer (Agilent, United States) with an Optima-17 column (30 m × 0.25 mm, 0.25 μm) from Macherey-Nagel (Germany). Injector temperature 280 °C; quadrupole temperature 150°C; ion source temperature 230 °C; type of ionization - electron impact; ionization energy 70 eV; sample volume 2 μl in 5:1 split mode. Division 12.691 cm3/min. The temperature of the GC-MSD interface unit 310 °C; column flow 3.02 ml/min. Chromatography was carried out under column heating conditions from 45°C (hold for 3 min) to 310°C (hold for 15 min) at a rate of 7°C/min. Total analysis time 56 min. Peaks were detected in the m/z range of 40–500. Mass spectra were identified using the NIST Mass Spectral Search Program for the NIST Mass Spectral Library (V. 2.2) software.

Table 1 shows the masses of samples of the drug Trametin Plus. The extractants, sample preparation method, analyzed sample components are indicated.

Sample number	***Preparation, mass, g***	***Analyzed components***	***Extractant***	***Features of sample preparation***
1.1	0.996	Common FAs		Acid hydrolysis of OFA
1.2	1.006			
2.1	1.013	Free FAs	Folch mixture for lipid extraction and n-hexane for FAME extraction	Alkaline esterification of FFA and extraction of ME FFA with n-hexane
2.2	1.024			
3.1	1.013	Esterified FAs		Transesterification of FFAs at pH = 6 and extraction of ME FFAs with n-hexane
3.2	1.024			
4	5.031	Non-polar components	*N-hexane*	Liquid-Liquid Extraction
5	5.025	Polar components	Ethanol	SPE on cartridges with RP-sorbent C18 and elution with ethanol
6	5.032	Polar components	Methylene chloride	Liquid-liquid extraction at pH 2.0

The Discussion of The Results

When analyzing free fatty acids (FFA), esterified fatty acids (EFA) and total fatty acids (TFA) of the veterinary drug Trametin Plus in the form of their methyl esters (ME). Among them, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were analyzed.

On the chromatograms of ME FFA extracts, 15 fatty acid peaks were identified, of which all 15 peaks were identified. On the chromatograms of extracts of ME FFA, 18 peaks of fatty acids were identified, of which all 18 peaks were identified. The chromatograms of extracts of ME OFA showed 18 fatty acid peaks, of which all 18 peaks were identified. In total, 18 fatty acids were identified in the samples of the veterinary drug Trametin Plus. Of these, 12 saturated fatty acids, 5 monounsaturated fatty acids and one polyunsaturated essential ω-6 polyunsaturated linoleic acid, presented in minimal quantities. Chromatographic characteristics of identified acids are presented in Table 2, qualitative composition and

quantitative composition - in Table 3.

the preparation in a bound form as acid residues of triglycerides and phospholipids accounts for 45.0% of all fatty acids of the preparation. The share of free unbound fatty acids accounts for 55.0% of all fatty acids of Trametin Plus (Table 3).

The share of esterified acids, that is, acids that are in

Table 2: Retention time tR and calibration coefficients k of identified FAs

Seq. No	*FA Formula*	*IUPAC name (and trivial name)*	t_R, *min*	*k*
1	12:0	Dodecanoic acid (lauric)	27.603	1.2149
2	13:0	Tridecanoic acid (tridecyl)	33.433	1.2416
3	iso-14:0	Iso-tetradecanoic acid	36.662	1.3677
4	a/iso-14:0	Anti-iso-tetradecanoic acid	36.950	1.3677
5	14:0	Tetradecanoic acid (myristic)	39.098	1.3677
6	iso-15:0	Iso-pentadecanoic acid	42.492	1.3842
7	a/iso-15:0	Anti-iso-pentadecanoic acid	42.975	1.3842
8	15:0	Pentadecanoic acid (pentadecylic)	44.562	1.3842
9	16:1	Cis-11-hexadecenoic acid	48.364	1.2873
10	16:1	Cis-9-hexadecenoic acid (palmitoleic)	48.522	1.2873
11	16:0	Hexadecanic acid (palmitic)	49.880	1.6130
12	17:0	Heptadecenoic acid (margaric)	52.928	1.1129
13	18:2	Cis, cis-9, 12-octadecadienoic acid (linoleic)	57.902	1.2477
14	18:1	Cis-11-octadecenoic acid (cis-vaccenic)	58.240	1.5533
15	18:1	Cis-9-octadecenoic acid (oleic)	58.535	1.5533
16	18:0	Octadecanoic acid (stearic)	59.686	1.7802
17	20:0	Eicosanoic acid (arachidic)	68.757	1.8832
18	22:1	cis-13-docosenoic acid (erucic)	76.026	1.6634

The main share of all fatty acids of the drug (82.0%) falls on saturated fatty acids. The share of monounsaturated fatty acids accounts for 16.0% of all acids, the share of polyunsaturated fatty acids is 2.0% (Table 3). There is a redistribution in the composition of fatty acids depending on the degree of their saturation between free and bound acids. So, for free acids, the share of saturated fatty acids is 67.0%, and the share of unsaturated acids is 33.0%; for bound acids, saturated fatty acids account for 82.0%, while unsaturated acids account for 18.0%. The predominance of unsaturated acids in the free non-esterified form was noted (Table 3).

Table 3 shows that the average concentration of fatty acids in the drug Trametin plus is about 70.0 mcg/g of the veterinary drug. The concentration of saturated fatty acids is about 57.0 mcg/g. The concentration of unsaturated fatty acids is about 12.0 mcg/g. The concentration of free fatty acids is about 41.0 mcg/g, the concentration of esterified fatty acids is about 34.0 mcg/g of the preparation.

Table 3: *Qualitative and quantitative (mcg/g) composition of fatty acids of Trametin Plus*

FA Formula	**The content of fatty acids in the preparation, mcg/g of the preparation**									
	Sample numbers						***Average content***			
	1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**TFA[1]**	**EFA**	**FFA**	**TFA[2]**
12:0	0.099	0.098	0.091	0.127	0.031	0.709	0.099	0.109	0.370	0.478
13:0	0.122	0.121	0.000	0.000	0.045	0.145	0.121	0.000	0.095	0.095
iso-14:0	0.444	0.439	0.226	0.237	0.280	0.696	0.441	0.232	0.488	0.720
a/iso-14:0	0.234	0.232	0.299	0.274	0.067	0.146	0.233	0.287	0.106	0.393
14:0	3.317	3.284	1.107	1.031	0.890	3.325	3.301	1.069	2.108	3.177
iso-15:0	3.835	3.797	6.659	5.886	0.132	0.451	3.816	6.272	0.291	6.564
a/iso-15:0	6.221	6.159	10.26	9.481	0.252	0.984	6.190	9.871	0.618	10.49
15:0	1.642	1.626	0.290	0.320	0.363	1.238	1.634	0.305	0.800	1.106
16:1	2.951	2.921	0.852	0.810	1.601	3.397	2.936	0.831	2.499	3.330
16:1	1.090	1.079	1.629	1.287	0.426	0.621	1.085	1.458	0.524	1.982
16:0	28.57	28.29	8.270	7.760	7.33	23.57	28.428	8.016	15.45	23.46
17:0	1.077	1.066	1.365	1.061	0.193	0.164	1.071	1.213	0.179	1.392
18:2	1.161	1.150	0.555	0.505	0.932	1.771	1.156	0.530	1.352	1.881
18:1	6.047	5.987	2.038	1.878	4.801	10.37	6.017	1.958	7.586	9.544
18:1	0.550	0.544	0.318	0.289	0.367	0.830	0.547	0.304	0.598	0.902
18:0	10.71	10.60	1.691	1.580	3.330	9.605	10.65	1.636	6.468	8.103
20:0	0.735	0.727	0.000	0.000	0.177	0.413	0.731	0.000	0.295	0.295
22:1	0.589	0.583	0.000	0.000	0.834	1.288	0.586	0.000	1.061	1.061
Total	69.39	68.70	35.65	32.53	22.05	59.72	69.05	34.09	40.88	74.97
SFA	57.00	56.44	30.26	27.76	13.09	41.44	56.72	29.01	27.26	56.27
MUFA	11.23	11.11	4.838	4.265	8.030	16.51	11.17	4.551	12.27	16.82
PUFA	1.161	1.150	0.555	0.505	0.932	1.771	1.156	0.530	1.352	1.881

Note: TFA[1] is the total content of fatty acids (free and esterified) obtained by a direct method (see point 2); TFA[2] is the total content of fatty acids (free and esterified), obtained by summing the values of the contents of FFA and EFA in the samples obtained according to point 3.

The percentage of fatty acids is shown in Table 4.

Qualitative analysis of volatile polar and non-polar organic compounds of the veterinary drug Trametin Plus was carried out using the NIST Database mass spectrum library. A very complex composition of the components is noted. In particular, this applies to hexane and ethanol extracts of the drug. A significant portion of the peaks in the chromatograms are inhomogeneous peaks that cannot be identified by simple comparison with the NIST mass spectra libraries. A significant part of the peaks is recorded as homogeneous peaks, but also cannot be identified by comparing the mass spectra of these peaks with the library ones since the mass spectra of the peaks of Trametin Plus and the library spectra have significant differences.

Table 4: Qualitative and quantitative (% of the total) composition of the fatty acids of Trametin Plus

FA Formula	**composition of FA in the preparation, % of the amount of FA in the sample**									
	Sample numbers						***Average content***			
	1.1	***1.2***	***2.1***	***2.2***	***3.1***	***3.2***	***TFA[1]***	***EFA***	***FFA***	***TFA[2]***
12:0	0.14	0.14	0.25	0.39	0.14	1.19	0.14	0.32	0.90	0.64
13:0	0.18	0.18	0.00	0.00	0.20	0.24	0.18	0.00	0.23	0.13
iso-14:0	0.64	0.64	0.63	0.73	1.27	1.17	0.64	0.68	1.19	0.96
a/iso-14:0	0.34	0.34	0.84	0.84	0.30	0.24	0.34	0.84	0.26	0.52
14:0	4.78	4.78	3.11	3.17	4.04	5.57	4.78	3.14	5.15	4.24
iso-15:0	5.53	5.53	18.7	18.1	0.60	0.76	5.53	18.40	0.71	8.75
a/iso-15:0	8.96	8.96	28.8	29.2	1.14	1.65	8.96	29.0	1.51	14.0
15:0	2.37	2.37	0.81	0.98	1.65	2.07	2.37	0.90	1.96	1.47
16:1	4.25	4.25	2.39	2.49	7.26	5.69	4.25	2.44	6.11	4.44
16:1	1.57	1.57	4.57	3.96	1.93	1.04	1.57	4.28	1.28	2.64
16:0	41.2	41.2	23.2	23.9	33.2	39.5	41.2	23.5	37.8	31.3
17:0	1.55	1.55	3.83	3.26	0.88	0.27	1.55	3.56	0.44	1.86
18:2	1.67	1.67	1.56	1.55	4.23	2.97	1.67	1.55	3.31	2.51
18:1	8.71	8.71	5.72	5.77	21.8	17.4	8.71	5.74	18.6	12.7
18:1	0.79	0.79	0.89	0.89	1.67	1.39	0.79	0.89	1.46	1.20
18:0	15.4	15.4	4.74	4.86	15.1	16.1	15.4	4.80	15.8	10.8
20:0	1.06	1.06	0.00	0.00	0.80	0.69	1.06	0.00	0.72	0.39
22:1	0.85	0.85	0.00	0.00	3.78	2.16	0.85	0.00	2.60	1.42
Total	100	100	100	100	100	100	100	100	100	100
SFA	82.2	82.2	84.9	85.3	59.4	69.4	82.2	85.1	66.7	75.1
MUFA	16.2	16.2	13.6	13.1	36.4	27.6	16.2	13.4	30.0	22.4
PUFA	1.67	1.67	1.56	1.55	4.23	2.97	1.67	1.55	3.31	2.51

Most likely this is due to the complexity of the object and the uniqueness of the biological compounds that make up its composition, some of which may yet be simply unknown to science. Detailed identification of the components of the drug requires serious long-term scientific research, thorough and detailed sample preparation for each class of compounds, study of the structure of compounds using various methods of chromatography, mass spectrometry, nuclear magnetic resonance, etc. A number of peaks of volatile components in the chromatograms of these extracts can be identified with a probability of ≥70.0%. These compounds are presented below (Tables 5, 6).

Non-polar volatile components of the sample, soluble in n-hexane, are represented by compounds with small molecular weights Mr ≤350 (Table 5). Among them are pyrans, furans, alcohols, aldehydes, ketones, heterocyclic compounds, saturated aliphatic hydrocarbons (saturated n-alkanes), unsaturated diene hydrocarbons, including squalene, aromatic compounds, indole (Table 5)

The polar volatile components of the sample, soluble in ethyl alcohol, are represented by compounds with small molecular weights Mr ≤310 (Table 6). Among them, acetic acid, acetoin - one of the products of butanediol fermentation, acetic anhydride, furfural - a product of xylose dehydrogenation (Table 6), heterocyclic compounds furanone and pyranone, aldehydes, including phenylacetaldehyde, peptides, ketones, simple sugars and amino acids, aromatic compounds.

Table 5: Non-polar organic components of the drug Trametin Plus according to the NIST library

Seq. No	*t_R, min*	*Formula*	*Name according to NIST mass spectrum library*
1	2.089	$C_6H_{12}O$	Furan, tetrahydro-2,5-dimethyl-
2	2.204	$C_6H_{12}O$	2H-Pyran, tetrahydro-2-methyl-
3	2.239	C_7H_{14}	Cyclohexane, methyl-
4	2.647	$C_6H_{14}O$	3-Pentanol, 3-methyl-
5	2.941	C_7H_8	Toluene
6	3.336	$C_6H_{12}O$	3-Methyl-1-penton-3-ol
7	3.409	$C_6H_{14}O$	2-Hexanol
8	3.723	$C_6H_{12}O$	3-Hexanone
9	5.427	$C_7H_{16}O_2$	2-Pentanol, 5-methoxy-2-methyl-
10	5.507	$C_6H_{14}S$	2-Pentanol, 5-methoxy-2-methyl-
11	5.950	$C_9H_{18}O_3$	2-Ethoxyethyl 3-methyl butanoate
12	6.443	$C_6H_{10}O_2$	Ethanone, 1-(3-ethyloxiranyl)-
13	7.654	$C_{17}H_{30}O_4$	Oxalic acid, cyclohexyl nonyl ester
14	9.123	$C_{13}H_{26}$	methylcyclohexane
15	10.239	C_8H_8O	Benzeneacetaldehyde
16	12.882	$C_{11}H_{16}O$	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-
17	14.478	C_7H_5NS	1,2-Benzisothiazole
18	15.691	$C_{10}H_{12}O$	Anethole
19	15.776	C_8H_7N	Indole
20	17.211	$C_{14}H_{30}$	Tetradecane
21	21.004	$C_{16}H_{34}$	Hexadecane
22	21.477	$C_{12}H_{14}O$	1-Penten-3-one, 4-methyl-1-phenyl-
23	22.957	$C_{13}H_{16}O$	1-Hexen-3-one, 5-methyl-1-phenyl-
24	25.027	$C_{17}H_{34}O_2$	i-Propyl 12-methyl-tridecanoate
25	25.600	$C_{18}H_{38}O$	1-Octadecanol
26	26.382	$C_{16}H_{22}O_4$	Diisobutyl phthalate
27	27.515	$C_{17}H_{36}$	Tetradecane, 2,6,10-trimethyl-
28	27.929	$C_{16}H_{22}O_4$	Dibutyl phthalate
29	28.711	$C_{20}H_{40}$	1-Eicosene
30	31.553	$C_{22}H_{46}O$	Behenic alcohol
31	35.434	$C_{24}H_{38}O_4$	Diisooctyl phthalate
32	37.738	$C_{30}H_{50}$	Squalene
33	38.435	$C_{22}H_{43}NO$	trans-13-Docosenamide

Table 6: Polar organic components of the drug Trametin plus according to the NIST library

Seq. No	*t_R, min*	*Formula*	*Name according to NIST mass spectrum library*
1	2.586	$C_2H_4O_2$	Acetic acid
2	3.485	$C_4H_8O_2$	Acetoin
3	4.09	$C_4H_{10}O_2$	2,3-Butanediol, [S-(R*, R*)]-
4	4.489	$C_4H_6O_3$	Acetic anhydride
5	5.156	$C_5H_4O_2$	Furfural
6	8.046	$C_4H_4O_2$	2(5H)-Furanone
7	8.616	$C_6H_6O_2$	2-Furancarboxaldehyde, 5-methyl-
8	10.255	C_8H_8O	Benzeneacetaldehyde
9	10.932	$C_5H_{10}N_2O_3$	Glycylsarcosine
10	11.711	$C_6H_8O_4$	Pyranone
11	12.161	$C_{12}H_{14}N_4O_4$	2-Vinyl-9-[3-deoxy-β-d-ribofuranosyl] hypoxanthine
12	12.97	$C_{25}H_{44}N_2O_5S$	2-Myristynoyl pantetheine
13	13.712	$C_6H_{14}N_4O_2$	Arginine
14	14.534	$C_6H_6O_3$	5-Hydroxymethylfurfural
15	14.863	$C_8H_{14}O_7$	6-Acetyl-β-d-mannose
16	15.785	C_8H_7N	m-Aminophenylacetylene
17	20.963	$C_{19}H_{15}N_3O$	Imidazolo[1,2-a]pyridine-6-carbonitrile, 1,2,3,5-tetrahydro-7-methyl
18	24.782	$C_5H_4N_4O_3$	Uric acid
19	28.693	$C_{14}H_{22}N_2O_2$	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine
20	31.316	$C_{12}H_{14}N_2O_2$	2,5-Piperazinedione, 3-methyl-6-(phenylmethyl)-
21	31.714	$C_{11}H_{12}N_2O_2$	2,5-Piperazinedione, 3-(phenylmethyl)-
22	33.951	$C_{15}H_{20}N_2O_2$	Cyclo-(l-leucyl-l-phenylalanyl)
23	34.714	$C_{14}H_{16}N_2O_2$	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-

Volatile amino acids of Trametin Plus are presented as minor components of the sample. Among them are glycine and sarcosine in the form of the dipeptide glycylsarcosine, where sarcosine is a methyl

derivative of the amino acid glycine, arginine, and β-alanine, presented in the form of an amide of the amino acid β-alanine and pantoic acid (group B vitamin).

Among these compounds, there are compounds that have undeniable and sufficiently strong biological activity. Activity is manifested as anti-inflammatory, antibacterial, antiviral, antioxidant, enveloping, immunomodulatory, and antitumor effects. At the same time, a number of compounds have undoubted toxicity in animal experiments, according to the literature data, including hepatotoxicity, carcinogenicity, general toxic effect, and neurotoxicity.

Conclusion

A quantitative analysis of fatty acids of the veterinary drug Trametin Plus was carried out. It is shown that the total concentration of fatty acids is 70 μg/g of the preparation. Of these, 50.0% are free fatty acids, most of which have a powerful antibacterial, antiviral, and antitumor effect, while esterified (bound) acids, which account for 50.0% of the total mass of fatty acids, to a greater extent, have an enveloping effect. and anti-inflammatory action. At the same time, essential polyunsaturated fatty acids are practically absent in the preparation. According to the literature, a high content of free fatty acids can be characteristic of a number of algae [6], micellar fungi [7], and their analysis plays an important role in diagnostic medicine [8].

Among the dominant non-polar volatile components of the drug Trametin Plus there is a triterpene compound - squalene C30H50 (reliability of determination by GC-MS method ≥95.0%). This substance has a powerful antioxidant, immunostimulating, enveloping surface-active effect, protecting the mucous membranes of the body.

The simplest organic aliphatic amino acids such as glycine (M = 75 g/mol), arginine (M = 174 g/mol), β-alanine (M = 89 g/mol) with a small molecular weight are presented as minor non-polar volatile components of Trametin plus.

The remaining organic components of the drug are able to have a complex effect on the body. The toxicity of some components to animals may be considered to establish the doses and timing of the use of the drug Trametin Plus.

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