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RESEARCH ON STEROIDS

This article examines the research methods on nandrolone and methandienone. The most sensitive reagents were selected to differentiate and identify these drugs. Medicinal products were identified by gas chromatography methods with mass-selective detector and gas chromatography method with PID detector. In addition to determining the qualitative characteristics of medicinal products by means of gas chromatography with PID detector, a quantitative analysis of the test substances was carried out. The main absorption bands characteristic of the investigated medicinal products was determined by IR spectroscopy.

In this regard, the development of effective approaches to forensic research and methods of determination on potent anabolic steroids in various objects of forensic chemical examination with the use of thin-layer chromatography, gas chromatography with mass-selective and flame-ionization detectors is relevant as the risks of their abuse in the illegal market and the availability of counterfeit products (with unknown active substance content) on the legal market are not excluded.

Several methods that are most effective for use while examining materials, substances and products include various types of chromatography (TLC, GC, GC-MS) in combination with the method of IR-spectroscopy, provide an opportunity to establish the structure of the analyte and identify it, and determine the concentration. The predominance of these methods is in simplicity and expressiveness. They do not require special reagents and solvents, there is no complex sample preparation for them, and their definition is not affected by fillers, such as sugar, starch, stearates and sodium carbonate.

Thus, because of several studies of anabolic steroids of nandrolone and methandienone by different physical and chemical methods, we can conclude that the complex application of these methods allows to conduct expert research, to determine the qualitative and quantitative composition of substances and mixtures, as well as to solve diagnostic and identification issues.

Keywords: nandrolone, methandienone, thin-layer chromatography, IR-spectrometer, gas chromatography.

Formulation of Research Problem. The definition of controlled substances and drugs in the objects of the forensic chemical (forensic) examination is one of the main tasks solved by the forensic centers of the Ministry of Internal Affairs.

Anabolic steroids are referred to as a class of pharmacological drugs that, in chemical structure and pharmacological action, are close to testosterone and are its derivatives with a weakened androgenic and enhanced anabolic effect. Almost all of them have a common mechanism of pharmacological action. Anabolic steroids increase the plastic and energy processes in the human body, thus increasing the function of cellular respiration and oxygen transport function of the blood, as the total amount of blood and erythrocytes increases. Unlike corticosteroids, anabolic steroids reduce platelet aggregation and adhesion, as well as blood clotting, which improves microcirculation during intense exercise.

The widespread use of testosterone in drug therapy was hampered by its pronounced androgenic action. In this regard, steroid compounds, similar in structure to testosterone, or having selective anabolic activity with less pronounced androgenic action were synthesized.

Analysis of Current Researches. In Ukraine, some of the anabolic steroids are under national control and are classified as potent. Effective approaches to forensic research and methods for identifying potent anabolic steroids have been virtually not considered. Partial research on steroid hormones are given in the works of S. M. Biryukov, N. A. Fitseva¹, and I. N. Fitseva.²

In this regard, the development of effective approaches to the forensic investigation of methods for determining potent drugs using thin layer (TLC), gas chromatography with mass selective (GC-MS), flame ionization (GC-FID) detectors, infrared (IR) spectroscopy is relevant, since both the threat of abuse in the illegal market of controlled substances and the appearance of falsified products (with an unknown content of the

¹ Biriukov S. M., Vartuzov V. V., Minin O. V. Doslidzhennia sylnodiiuchykh likarskykh zasobiv, shcho mistiat metandiienon i nandrol on dekanat v ob'iektakh kryminalistychnoi ekspertyzy [investigations of potent drugs containing methandienone and nandrol decanate in objects of forensic examination]. URL : <http://elar.naiu.kiev.ua/jspui/handle/123456789/2070> [in Ukrainian].

² Ficev I. M., Blohin V. K., Budnikov G. K. Hromatografichskie metody v kriminalisticheskoi jekspertize [Chromatographic methods in forensic examination]. Zhurn. analit. himii. 2004. T. 59, № 12. S. 1289–1299 [in Russian].

current principle) in the legal headlight market are not excluded medications.

Research Purpose. The use in medicine, the available cases of poisoning, as well as attempts to partially study steroids, the lack of guidelines for the study of qualitative and quantitative determination in expert organizations is one of the pressing problems of modern chemical analysis.

The purpose of this article is to select the most sensitive reagents and systems for thin-layer chromatography, as well as the conditions for conducting gas chromatography with mass-selective detection for qualitative determination and quantitative analysis by gas chromatography, as well as IR spectrometers.

Main content presentation. Methods that are most effective for use in the examination of materials, substances and products include various types of chromatography (TLC, GG, GC – MS), in combination with IR spectroscopy, make it possible to establish the structure of the test substance and identify it, as well as determine the concentration. Priority of these methods is simplicity and expressiveness. They do not require special reagents and solvents, they do not have a stage of complex sample preparation, excipients (sugar, starch, stearate and soda) do not affect their determination. Therefore, there is a need to create sensitive and low-cost research methods for the drugs in this group to reduce the timing and rapid response to chemical research. As for the representatives of this group, they are increasingly becoming the objects of study of chemical toxicological and chemical laboratories, since their sale is controlled in the territory of Ukraine and is subject to substantive quantitative accounting in the pharmacy network. Relatively inexpensive but fairly accurate method of investigation is thin layer chromatography (TLC). The chromatographic characteristics were determined in a variety of organic solvents.

Sorbfil plates were used for TLC. As reagents, developers used Marki's reagent and iodine vapors. In the study of objects for belonging to high-potency anabolic steroids by TLC, chromatographic systems known in the art were used, with mobile phases characterized by efficiency and selectivity of separation, relative accessibility and not high toxicity.

Composition of the mobile phases was selected in such a way that the results obtained were as effective as possible and well reproduced.

Thus, TLC systems were selected, the composition of which is shown in Table 1.

Table 1

The composition of the mobile phases for research by TLC method

System No.	Composition of the mobile phases
1	toluene – ethanol – diethylamine (9: 1: 1)
2	chloroform – acetone – ethanol – 25% aqueous ammonia solution (20: 30: 3: 1)
3	Methanol – 25% aqueous ammonia solution (100: 1, 5)
4	toluene – acetone – ethanol – 25% aqueous ammonia solution (45: 45: 7: 3)
5	ethyl acetate – isopropanol – 25% aqueous ammonia solution (40: 30: 2)
6	methylene chloride – ether – methanol – water (77: 15: 8: 1, 2)

Given that the objects under study are well soluble in various solutions, solvents such as chloroform, ethanol, and methanol were used to prepare the samples.

Under TLC conditions, 10 µl of ethanol extracts of the objects under study were microscopically applied to a Sorbfil PTH-AF-A-UV thin-layer chromatography plate. On the same plate in an amount of 10 µl were applied standard samples – 0, 1% ethanol solution of methandienone and nandrolone, as well as control samples of the solvent. The plate was placed in a chromatographic chamber saturated with one of the solvent systems and chromatographed in an ascending manner until the eluent front was lifted 100 mm. After the end of the chromatography, the plate was dried at 50°C for 10 min, and then chromatographic zones were detected by fluorescence quenching at 254 nm, while fixing their position. Subsequently, the identified chromatographic zones were shown by visualizing reagents. Visualization of the chromatographic zones was performed with a Marki reagent (1-part 40% formalin solution 9 parts concentrated sulfuric acid) and in an iodine chamber, with saturated iodine vapors. The results of the study by chromatography in a thin layer of sorbent using various mobile phases and methods of visualization of the chromatographic zones by reagents – developers are presented in Fig. 1 and Table 2.



(a)



(b)

Fig. Fig. 1 (a) is a general view of the chromatograms of TLC of methandienone in systems 1, 2, 3 after the manifestation of 254 nm in UV; (b) is a general view of the chromatograms of TLC of methandienone in systems 1, 2, 3 after the manifestation of 254 nm in UV; (b) is a general view of the chromatograms of TLC of methandienone in systems 1, 2, 3 after the manifestation of 254 nm in UV

Table 2

Chromatographic mobility (R_f) values of some potent anabolic steroids in chromatographic systems

№	Test substances	Chromatographic mobility (R_f) values of some potent anabolic steroids in chromatographic systems						Chromatographic zone staining	
		№1	№2	№3	№4	№5	№6	Mark reagent	iodine pairs
1	Metandienone	0, 56	0, 63	0, 71	0, 70	0, 65	0, 65	dark brown	yellowy-brown
2	Nandrolone	0, 44	0, 71	0, 71	0, 75	0, 67	0, 88	-	yellowy-brown

Analyzing the results of chromatographic mobility shown in the table, we can conclude that the most positive separation characteristics were obtained using systems 1, 2, 6. In this case, the values of R_f in all organic solvent systems used are close to each other, therefore, to increase the selectivity of this method, it is necessary to have samples for the comparative study of these substances. In addition to thin layer chromatography, it is advisable to use the molecular Fourier method of molecular spectral analysis among instrumental methods. However, as the practice shows, for the study of these substances come in the form of tablets and oily solutions, and along with the active substances may have fillers: sugar, starch, baking soda, etc., added to tablets and solutions in their manufacture. For isolating anabolic steroids for their further investigation by IR spectroscopy, they were extracted with organic solvents, then concentrated.

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spectrum registration range: 4000–550 cm^{-1} ;

Resolution: 4 cm^{-1} ;

number of scans– 32.

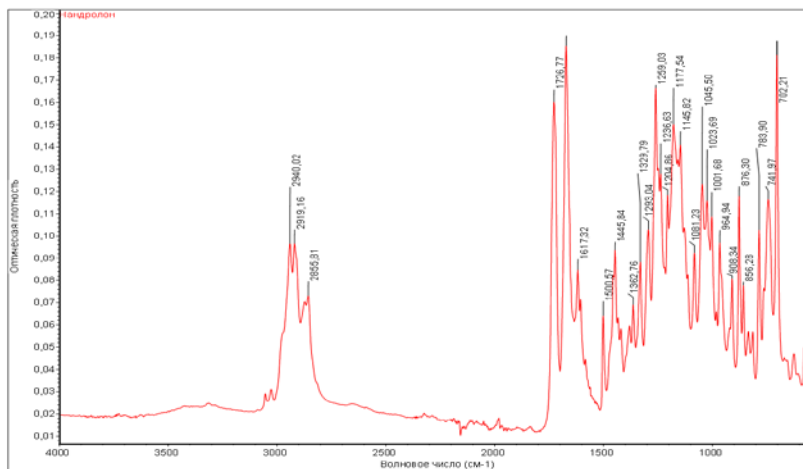


Fig. 2 IR spectrum of methanol extract of nandrolone

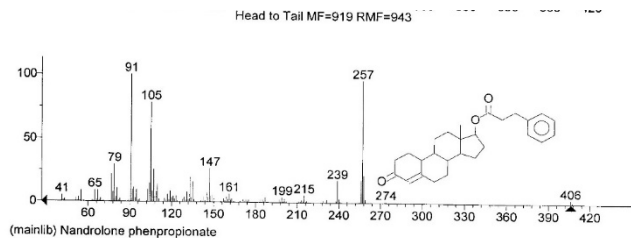
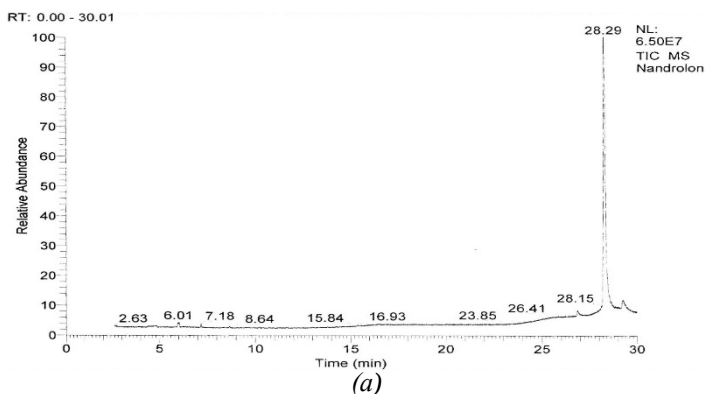
Decryption was performed on the library of IR spectra from the *Omnic 9.1* software package. For research clarity, to establish the molecular composition in Fig. 2 demonstrates steroid spectra.

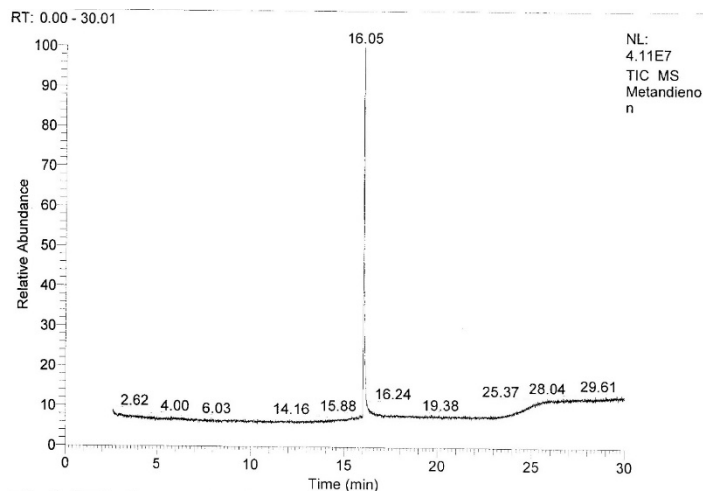
GC-MS object studies were performed to confirm the results of TLC analysis. The research was performed with the aim of qualitatively

determining the organic components in the research object. For this purpose, weighed portions of nandrolone and metandienone in weights of 5 mg each (ground medium sample) were dissolved in 1 ml of methanol.

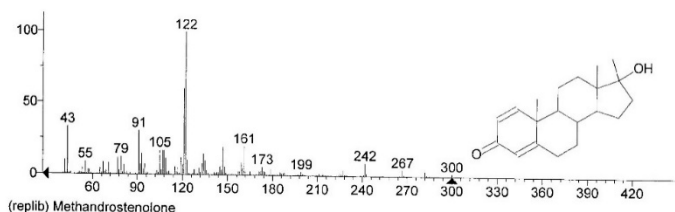
The obtained methanol extracts were filtered, transferred to glass vials and analyzed on a gas chromatograph with a selective detector with the conditions: TRASE ULTRA GC / ISQ / AS 3000 II; capillary column – TG – 5MS, length – 30 m, diameter – 0.25 mm, phase – 0.25 μ m; carrier gas – helium, constant flow – 1, 2 ml / min; injector – auto-injector 7683, Split 1:50, evaporator temperature $T = 280^{\circ}\text{C}$; column temperature: Tach. = 100°C , hold for 2 minutes, heating – $25^{\circ}\text{C} / \text{min}$, $T_{\text{кнц}} = 200^{\circ}\text{C}$, trim 2 xv; heating – $10^{\circ}\text{C} / \text{min}$, $T_{\text{конец}} = 280^{\circ}\text{C}$, hold – 7 min, heating – $10^{\circ}\text{C} / \text{min}$, $T_{\text{кон}} = 300^{\circ}\text{C}$, hold 5 min, detector – mass-selective, interface temperature $T = 220^{\circ}\text{C}$, ionization – by electron impact, ionization energy – 70 eV, temperature of the ion source $T = 200^{\circ}\text{C}$; quadrupole temperature $T = 150^{\circ}\text{C}$; sample – 1 μ l.

Chromatograms and mass spectra of nandrolone are presented in Figs. 3 a, b, methandienone – in Fig. 3 in, g.





(c)



(d)

Fig. 3 (a) chromatogram of a solution of nandrolone in methanol; (b) the mass spectrum of nandrolone; (c) a chromatogram of a solution of methanediene in methanol; (g) a mass spectrum of methanediene.

To separate the components of anabolic steroids, retention time was determined under conditions of GC – FID.

Quantitative determination of potent anabolic steroids under conditions of GC – FID was carried out according to the calibration dependence, using absolute calibration. As an external standard, a sample provided by SSRFC of the Ministry of Internal Affairs of Ukraine was used.

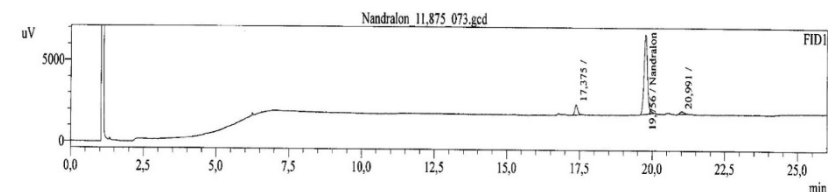
The basic requirements for reproducibility of GC – FID, as well as for GC – MS, are the accuracy of preparation of calibration solutions and the dosage of the sample, sample, as well as strict adherence to chromatographic conditions both during calibration of the device and in further quantitative determinations. The retention time of the studied potent anabolic steroids during their gas chromatographic study are shown in table 3.

Table 3

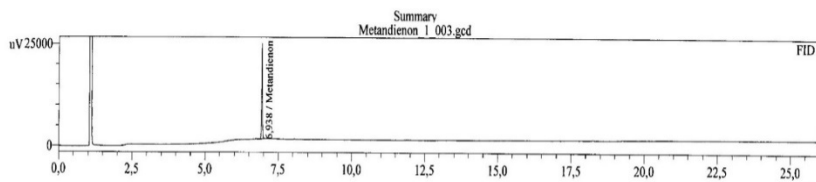
The retention time of the studied potent anabolic steroids during their gas chromatographic study are demonstrated in table 3.

№	Test substance	Retention time
1	Nandrolone	19, 756
2	Metandienone	6, 938

Chromatograms of nandrolone and methanedieneone obtained by chromatographic examination under GC – FID conditions are presented in Fig. 4 a, b.



(a)



(b)

Fig. 4 (a) a chromatogram of a solution of nandrolone in methanol, (b) a chromatogram of a solution of methandienone in methanol

Conclusions. Thus, during research on the anabolic steroids of nandrolone and methandienone by various physicochemical methods, it can be concluded that the combined use of these methods makes it possible to carry out their expert research, the qualitative and quantitative composition of the substances present in the mixture, and to solve diagnostic and identification problems.

References

- Bíryukov, S. N., Vartuzov, V. V., Minin, O. V. (2018). *Doslidzhennya synodiyuchykh likarskykh zasobiv, shcho mistyat metandiyenon i Nandrolon dekanat v ob'ektakh kryminalistychnoyi ekspertyzy*. URL: <http://elar.naiu.kiev.ua/jspui/handle/123456789/2070> [in Ukrainian].
- Fitsev, I. M., Blokhin, V. K., Budnikov, G. K. (2004). *Khromatografichskiy metody v kryminalisticheskoy ekspertize*. 12. Zhurn. analit. khimii. 1289–1299 [in Russian].

Mashkovskiy, M. D. (1986). *Lekarstvennyye sredstva*. Moskva: Meditsina. 604–606 [in Russian].

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ДОСЛІДЖЕННЯ СТЕРОЇДІВ

У статті розглянуто методи дослідження нандролону і метандієнону. Підібрано найбільш чутливі реактиви, які дозволяють диференціювати і ідентифікувати дані лікарські препарати. Проведено ідентифікацію лікарських засобів методами газової хроматографії з мас-селективним детектором та методом газової хроматографії з ПІД – детектором. Крім визначення якісних характеристик лікарських засобів методом газової хроматографії з ПІД детектором проводився кількісний аналіз досліджуваних речовин. Методом ІЧ- спектроскопії визначено основні смуги поглинання, характерні для досліджуваних лікарських засобів.

У зв'язку з цим розробка ефективних підходів до криміналістичного дослідження і методів визначення сильнодіючих анаболічних стероїдів у різноманітних об'єктах судово-хімічної експертизи із застосуванням тонкошарової хроматографії, газової хроматографії з мас-селективним та полумєново-іонізаційним детекторами, інфрачервоної спектроскопії є актуальною, оскільки не виключені як загрози зловживання ними на нелегальному ринку, так і поява фальсифікованої продукції (з невідомим вмістом діючої речовини) на легальному ринку фармпрепаратів.

До ряду методів, найбільш ефективних при застосуванні в криміналістичній експертизі, належать різні види хроматографії (ТШХ, ГХ, ГХ-МС), їх поєднання з методами ІЧ-спектроскопії дають можливість встановити структуру досліджуваної речовини та ідентифікувати її, а також визначити концентрацію. Перевагою цих методів являється простота і експресність. Вони не потребують наявності спеціальних реактивів та розчинників, для них може бути відсутня стадія складної пробопідготовки, а визначенню можуть не заважати наповнювачі, такі як цукор, крахмал, стеарати та сода.

Таким чином, у результаті проведення ряду досліджень анаболічних стероїдів нандролону та метандієнона різними фізико-хімічними методами, можна зробити висновок, що комплексне застосування цих методів дає можливість проводити експертне дослідження, визначати якісний та кількісний склад наданих речовин та сумішей, а також вирішувати діагностичні та ідентифікаційні завдання.

Ключові слова: нандролон, метадієнон, тонкошарова хроматографія, ІЧ- спектроскопія, газова хроматографія.

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