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Spectrum of analytical findings in chemical-toxicological examination of poisonings in the zone of a special military operation

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ABSTRACT

Introduction. The combat environment has determined the probability of toxicants' using in the special military operation zone, with respect to conventional restrictions and situational availability of materials and chemicals for the dispersion devices' loading. The importance of analytical indication of chemical products in the biological environment of the affected determines, on the one hand, the accuracy of diagnosis and, as a result, the adequacy of the prescribed therapy, and on the other hand, dictates the need to obtain irrefutable information about new tactics of using toxic chemicals as an alternative to chemical weapons.

Material and methods. Analytical studies of samples, including those obtained by the dried blood spot method, collected by medical specialists in outbreaks during the organization of medical evacuation and in first-level medical institutions, were conducted to characterize the spectrum of chemical safety threats.

Results. The spectrum of adverse chemical factors is associated with the possibility of implementation through criminal activity, as well as the use of unmanned gas-release and aerosol-generation vehicles. The threat of neurotropic agents is illustrated by the cannabinoids' use consisting of spices, substances of bioactive cardiotropic agents, soluble metal salts, including uranium, phosphothermite mixtures of abnormally detonated ammunitions. The greatest risk of using as means of destruction is realized by the police riot control supplies and irritants from among the compounds of industrial synthesis and solvents. The formation patterns of delayed tracheobronchial tree and lung tissue lesions caused by corrosive substances were associated with aerodynamic properties of the latter formulated into dry aerosols produced by explosive or knockout (propulsive?) generation from drones. The risks of natural poison threat materializing have been identified through the example of types A and B botulinum toxins but do not exclude extract using of poisonous plants growing on the territory of the warring parties.

Limitations. Analytical studies of samples do not apply to environmental objects, and the methodological possibilities of their interpretation are limited by the chemical nature of the analyte.

Conclusion. The spectrum of toxicants that had been used for several years of military confrontation reflected the possibilities of their situational production and use for aerosol-generation or gas-release devices' loading. It determines the aspects of providing medical care to the victims in the toxicogenic phase of intoxication.

Keywords: *cannabinoids; soluble metal salts; irritants; corrosives; detergents; toxins*

Compliance with ethical standards. The study was performed in compliance with the ethical principles of conducting medical research with the participation of people as subjects of research in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles of conducting Scientific medical research with human participation" as amended in 2013 and the "Rules of Clinical Practice in the Russian Federation", approved by Order of the Ministry of Health of the Russian Federation No. 266 dated 06/19/2003. All participants gave informed voluntary written consent to participate in the study.

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Introduction

The risks of military confrontation increase the likelihood of using chemical weapons, which, according to D.I. Mendeleev's figurative comparison, are referred to as "weapons of the poor" [1]. The introduction of conventional restrictions and their strict observance under threat of international censure, however, do not limit the possibility of using substances outside the conventional lists, dual-use agents, which, if properly shaped, can achieve significant effectiveness in defeating people [2]. Such products primarily include derivatives of natural raw materials, medicinal products, including narcotic and potent drugs, industrial reagents, detergents and fertilizers, rodenticides, etc. Examples of their use as weapons of destruction have been traced for police riot control equipment of various compositions and smoking mixtures (spices), mainly of a cannabinoid structure [3, 4].

All these compounds find their role and place in modern military confrontation, expanding the doctor's tasks in diagnosing the entire spectrum of diverse lesions, forming therapeutic tactics for their relief. At the same time, the assessment of chemical risks in relation to the defeat of people tends to aggrandize. Not all substances, even despite their obvious toxic properties, can be used as a means of destruction [5]. In this regard, the experience of military confrontation requires an analysis of chemical risks, a detailed study of the mechanisms of their implementation, ranking and formation of restrictions for the development of universal syndrome approaches to therapy and actions of doctors in the event of poisoning [6].

The purpose of this work was to describe the identified variants of toxic pathology, the etiology of which has been proven using analytical methods of studying the skin, skin appendages and the biological environment of the body.

Material and methods

The research material includes cases of poisoning with various substances in the zone of a special military operation. The condition of some of the victims was studied in the conditions of first-level medical institutions with the possibility of therapeutic and resuscitation measures, as well as in the conditions of the clinical center. In addition, samples were taken from the victims, including those who were not under observation, using dry drop sorption cartridges delivered to the Institute's laboratories from hospitals and medical institutions of the medical service. The applied sorption technologies ensured the transportation of samples of the biological environment and the environment for research on various analytes, including proteins, without the need to comply with the cold chain. The cartridges were manufactured and transferred to advanced medical institutions to form a chemical safety control system. Some of the samples were delivered in the traditional manner in test tubes. Upon receipt of the cartridges, sorbent stains with bioassays were cut out and immersed in organic solvents of a special composition for desorption, and various physico-chemical concentration methods were used to enrich the samples. After liquid-liquid extraction, the bioassays were concentrated by vacuum drying and subjected to analysis using standard algorithms on gas (Agilent, USA) and liquid (Thermo Fisher Scientific, USA) chromatomass spectrometers, as well as on inductively coupled plasma quadrupole mass spectrometer (Helicon 7000, China). A number of samples were examined by scanning electron microscopy using an EM8000 microscope (KYKY, China) under high vacuum conditions. An energy dispersion microanalysis system (AZtecOne, Oxford Instruments) was used to assess the elemental composition of the samples. Before conducting the study, the sample substrate was treated with gold particles (10 nm) using a sample vacuum spraying unit (DCR, Nano-Structured Coating).

When protein toxins were detected, a blood plasma sample in a test tube with a coagulation activator was prepared for analysis by HPLC-HRMS using ProteoMiner affinity spin columns (BioRad, USA) and using specific antibodies to botulinum toxin A and B. All samples were subjected to enzymatic cleavage with trypsin on centrifuge filters with a 50 kDa cutoff. The analysis was performed using standard samples of botulinum toxins A and B.

Results

With regard to medicinal, narcotic and psychotropic drugs, attempts have been traced to the aerosol application of substances of potent cardiotonics (including cardiac glycosides) and antiarrhythmic drugs on nano- and micro-sized carriers with characteristic manifestations of damage. Methadone and the stimulants mephedrone and α-pyrovaleron were most often detected in caches in the new territories of the Russian Federation. Among neurotropic drugs, attention was drawn to cannabinoid poisoning, which was traced in several clinical cases accompanied by temporary loss of consciousness. In each of them, on the 3rd–5th day after poisoning, 1H-indazole-3-carboxylic acid was detected in the urine of the victims (Fig. 1) as a metabolic product of indazole-3-carboxamide

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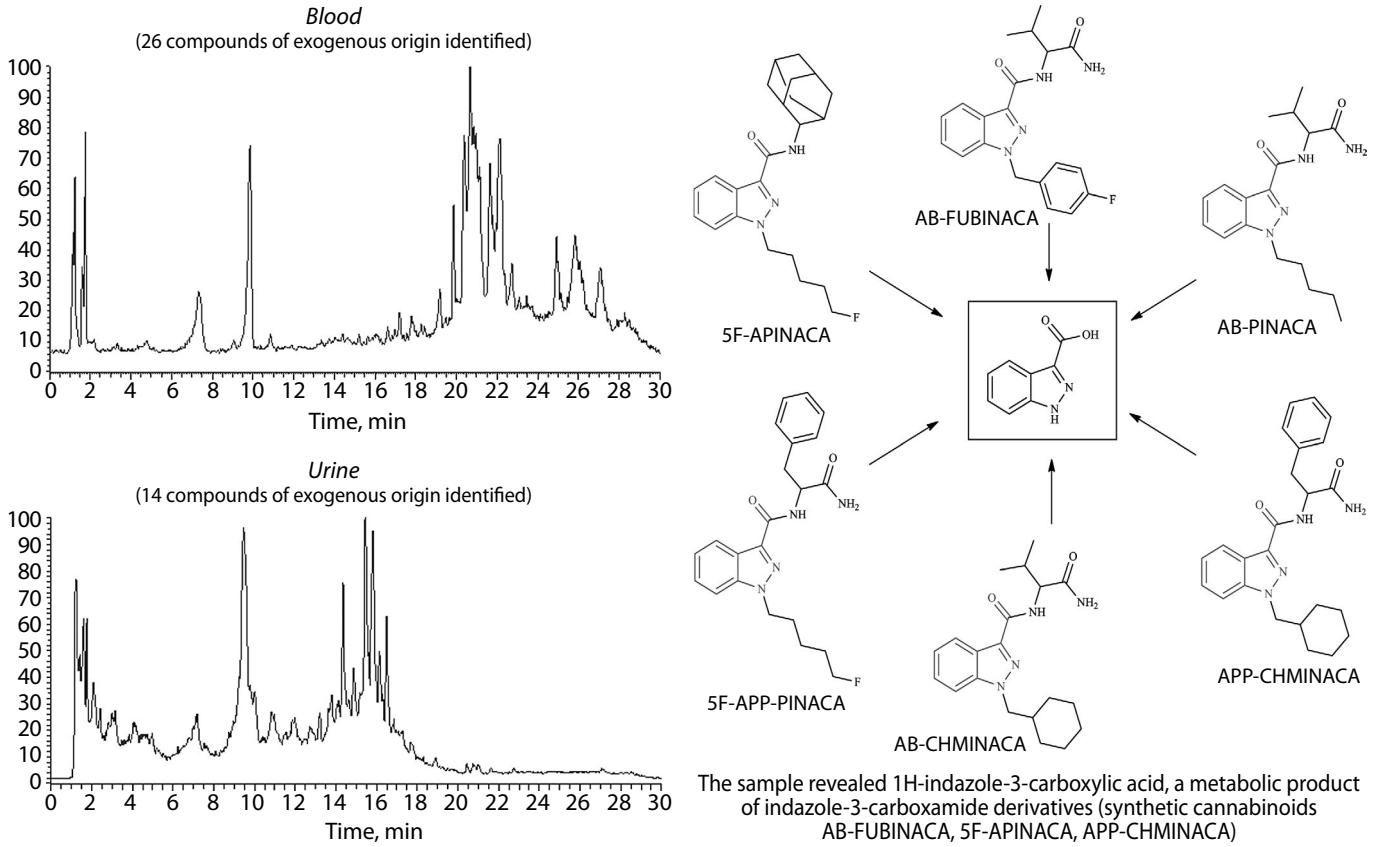

Fig. 1. Detection of cannabinoid metabolic products in the blood (*a*) and urine (*b*) of victims. OY axis – relative content, %.

derivatives (synthetic cannabinoids such as AB-FUBINACA, 5F-APINACA, APP-CHMINACA). The circumstances of poisoning and the type of metabolism indicate inhaled intake of cannabinoids due to criminal use.

Cases of food contaminated with soluble metal salts have been identified (Fig. 2). The presence of barium, cadmium and mercury has been traced in blood samples of the poisoned.

An important diagnostic finding was the detection of uranium in trace amounts in the hair of persons in the subclinical stage of poisoning (Fig. 3).

Such findings indicate the risks of ingestion of the products of the destruction of uranium cores of artillery ammunition [7]. Poisoning with phosphorus-thermite mixtures of improperly triggered artillery ammunition also became examples of damage by inorganic toxicants. Such sufferers with glowing

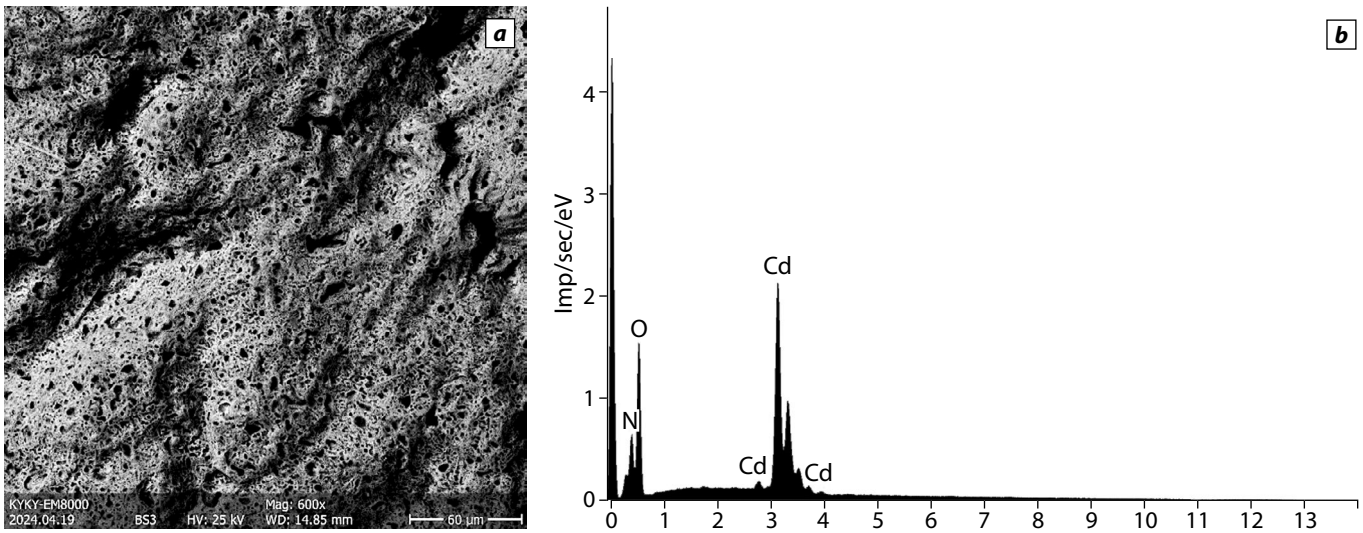

Fig. 2. Photomicrography of the cadmium nitrate's surface morphology found in foodstuff, magnification ×1000 (voltage pencil 25 kv) – *a* and Xray analysis results for the study sample's elementary composition (presented by N.G. Vengerovich) – *b*.

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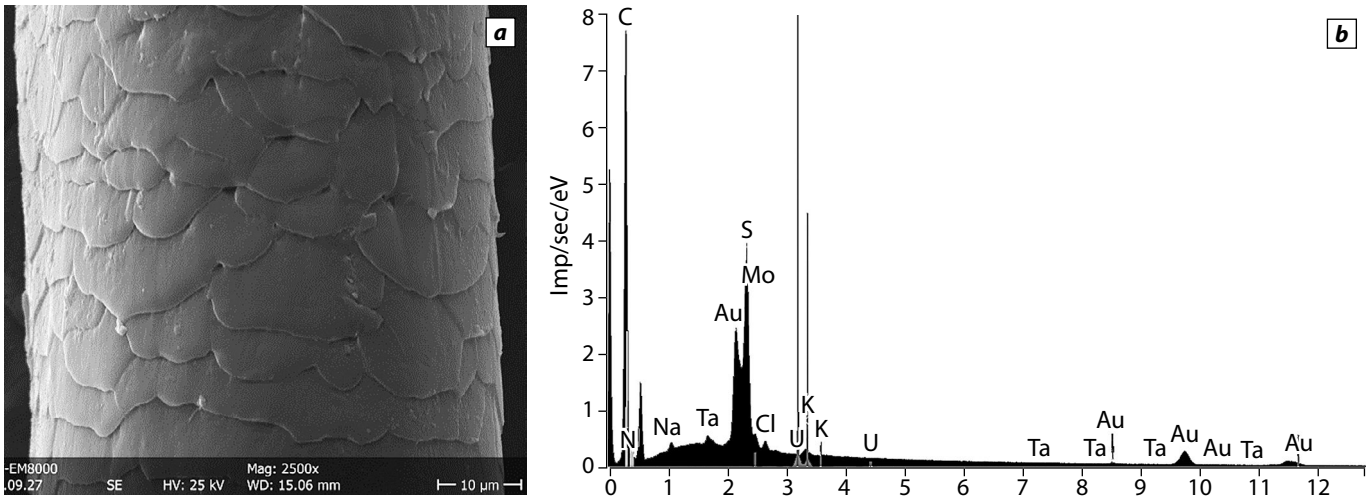

Fig. 3. Photomicrography (*a*) of the human hair surface found in foodstuff, magnification ×2500 (voltage pencil 25 kv), and Xray analysis results (*b*) for the study sample's elementary composition (presented by N.G. Vengerovich).

uniforms, characteristic poisoning clinic, blood changes and hepatopathies have been traced in a therapeutic hospital.

Essential for understanding the mechanism of delayed lung lesions with the formation of pulmonitis, some of which were fatal, was the detection of poisoning with organic highly alkaline detergents (including those containing mono- and triethanolamine and their derivatives) and detergents, the components of which penetrate into the blood [8] and which were identified during an analytical study (Fig. 4). After spraying with a UAV in the form of a dry aerosol, these irritating substances are washed off from hands and face (practically no eye damage

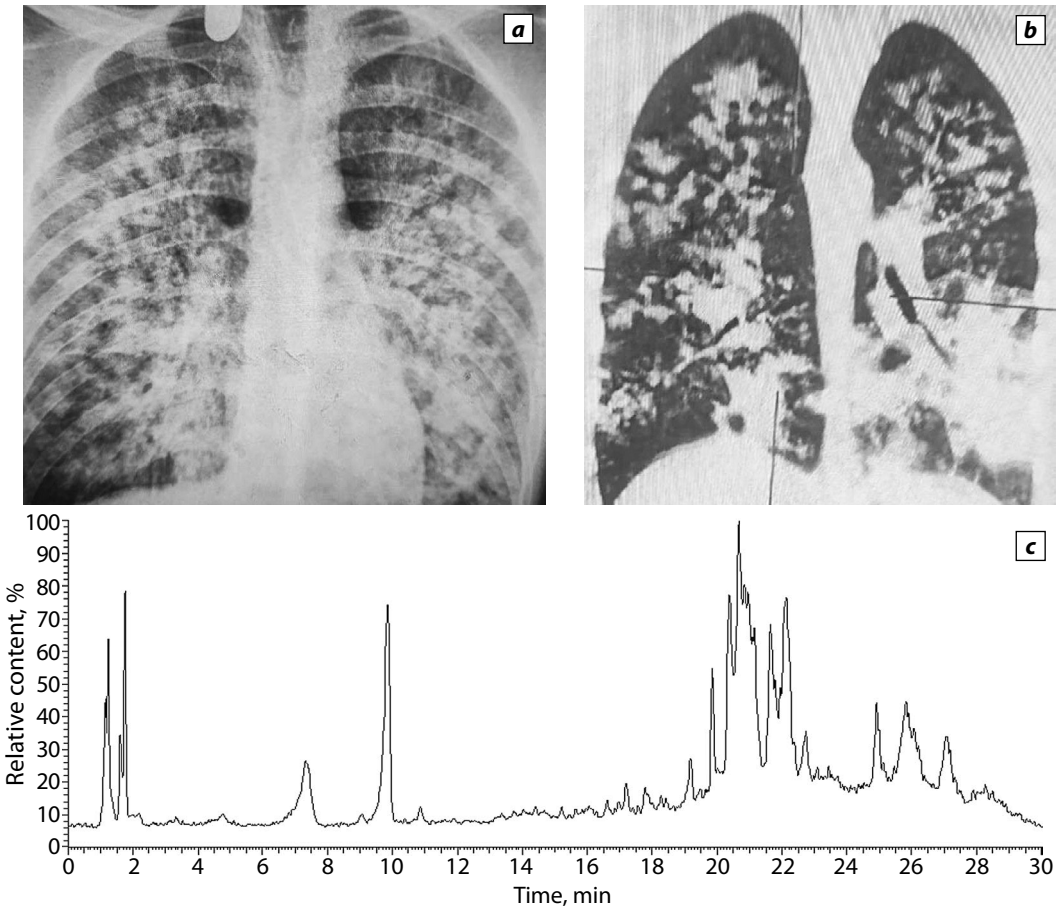

Fig. 4. X-ray manifestations of delayed pulmonitis during large-field fluorography (*a*) and computed tomography (*b*) studies (were presented by E.V. Raguzin) and the spectrum of components of dry alkalis and detergents resorbed into the blood that caused them (*c*).

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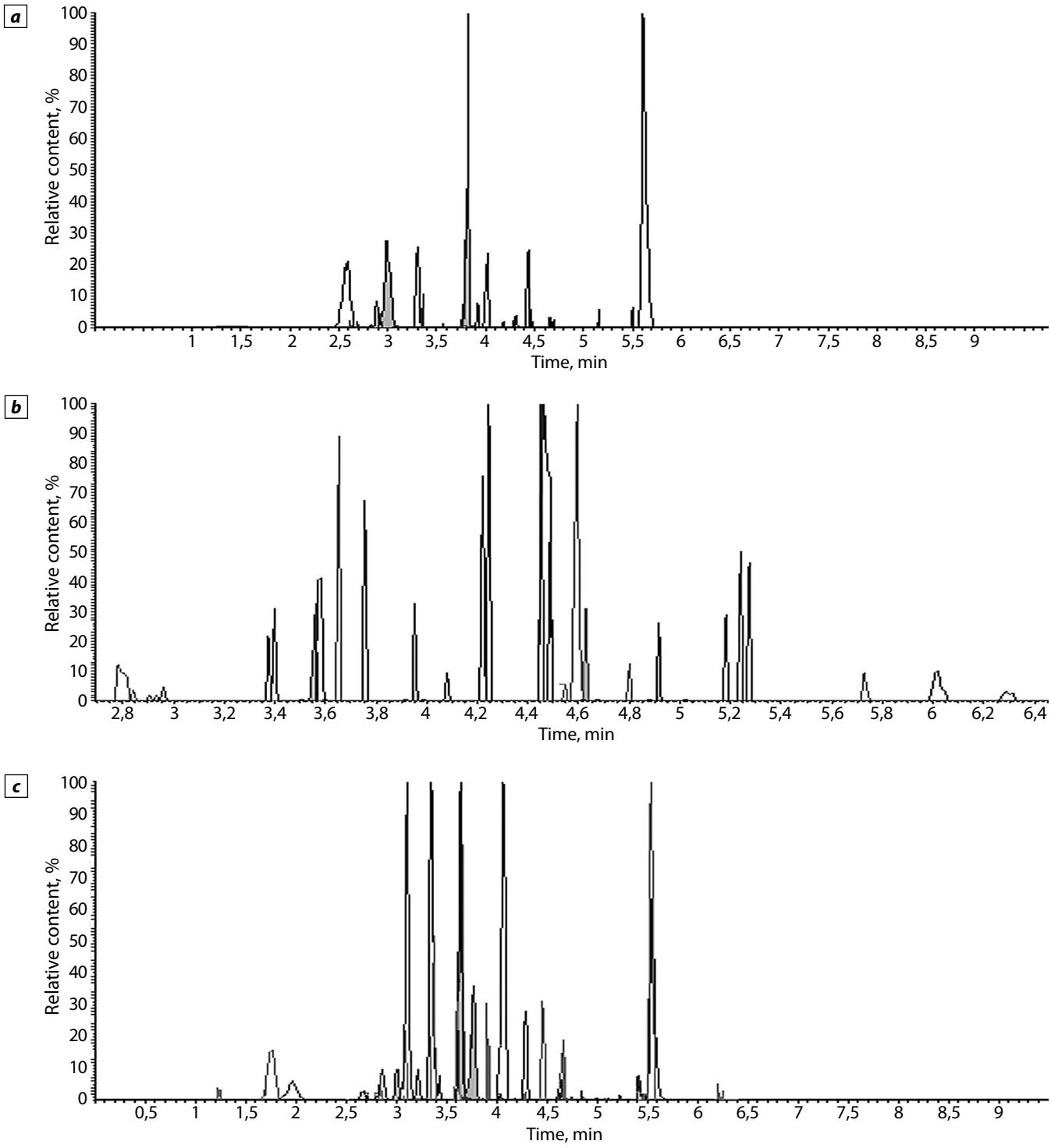

Fig. 5. Mass chromatograms of blood plasma samples purified using specific antibodies to botulinum toxin A (*a*) and botulinum toxin Б (*b*) and using affinity spin columns B (*c*), with peaks of characteristic peptides identical to control samples with added toxin samples (analysis performed by N.S. Yudina).

was detected after washing), however, the toxic agent continued to have a corrosive effect on the mucous membrane of the trachea and bronchi for several days, forming deep necrotic changes in both the walls of the airways and the adjacent lung tissue.

Similarly to organic alkalis and soaps, the use of tosyl chloride (4-toluenesulfonyl chloride) and other organic acids and oxidizing agents has been established for the equipment of dispersing devices. Their lesions were similar to alkali poisoning, but they manifested much faster and affected the larynx and trachea to a greater extent. It is advisable to include into the same group of alkylating poisons the slightly sweet-tasting liquefied fumigant ethylene

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oxide, which is widely used in various technological processes. The assumption of the presence of the substance was made after analyzing the complaints of the affected for a sweetish taste in the mouth and the presence of 2-chloroethanol [9] as a product of its interaction with organic molecules. Local and resorptive effects were observed with skin irritation and burns, as well as headaches, vomiting, and muscle twitching.

Police riot control agents (CS, pelargonic acid morpholide, chloroacetophenone) and other irritants – products of chemical production (chloroacetone, chloro- and bromocyanins, acrolein and other aliphatic aldehydes, sulfurous anhydride, chloropicrin, etc.) have found wide application as means of chemical destruction. Smoke detectors were used by the enemy as irritants. So, to generate smoke, UAVs are equipped with standard combat (M-18 smoke grenade) or modified airsoft smoke bombs, which include arsenic trioxide, hexachloroethane and hexachlorobenzene, naphthalenes, anthracenes (in particular 1-(methylamino) in various compositions, in addition to irritating nitrogen dyesanthracene-9,10-dione), phosphorus compounds, and other compounds, the sublimation or combustion of which can form a toxic aerosol. Only 16% of the poisoned people admitted to the first-level hospitals were able to describe the color of the smoke in detail: 59% of them described it as white, 19% as yellow, 13% as green, 6% as gray and 3% as purple-black. 39% of the victims could not clearly characterize the color of the aerosol they were in, and at least 30% described exposure to colorless gas without aerosol formation.

Among the means of chemical destruction, substances of natural origin were also used. Despite the availability of ricin from fruits growing in the *Ricinus communis* region, no poisoning has been detected in the territory of the combat zone. At the same time, the use of products contaminated with *botulinum toxin* has been traced. The studied proteins have regions with repeating amino acid sequences. For this reason, the purification of the target protein was carried out using antibodies to various types of toxin (Fig.5).

According to the results of the HPLC-HRMS analysis, it was found that botulinum toxin A is present in blood plasma samples, the characteristic peptides of which have a high signal intensity and are traced in the samples with all the sample preparation methods used.

It should be noted that, due to the region of growth, extracts of hogweed (*Heraculum* sp.), borsa (*Aconitum* sp.), hemlock (*Conium* sp.), hellebore (*Veratrium* sp.), foxglove (*Digitalis* sp.), lily of the valley (*Convallaria* sp.), poisonous mushrooms and others. However, obtaining active substances requires a high complexity and cost of extraction, purification and separation technologies, which minimizes the risks of their widespread criminal use in aerosols.

Discussion

The range of toxicants used during several years of military confrontation in the zone of a special military operation, on the one hand, reflects the possibilities of their situational acquisition, and on the other hand, deliberate use to equip aerosol generation or gas release devices. The frequent detection of amphetamine-type stimulants in caches in new territories of the Russian Federation, as well as the increased incidence of cannabinoid poisoning, indicate an intensification of criminal and sabotage activities. The circumstances of poisoning and the type of metabolites detected indicate an increase in cases of inhaled intake of cannabinoids, potent cardiotonics (including cardiac glycosides) and antiarrhythmic drugs on nano- and micro-sized carriers. The identified cases of food contaminated with soluble metal salts indicated the presence of barium, cadmium and mercury, which was traced in blood samples, as well as uranium in the hair of the poisoned persons. Examples of the latter can be poisoning with phosphorus-thermite mixtures of improperly triggered artillery ammunition.

A significant finding was the data on the identification of potent alkaline detergents (including those containing mono- and triethanolamine and their derivatives) and detergents that could be applied using UAVs in the form of a dry aerosol and required sanitation. The assumption of damage by the liquefied fumigant ethylene oxide was confirmed by the organoleptic sweet taste in the mouth and the presence of 2-chloroethanol [9] as a product of its interaction with organic molecules. These lesions were characterized by local and resorptive effects with skin irritation and burns, as well as headaches, vomiting, and muscle twitching.

In addition to the "classic" police riot control agents, the enemy used smoke detectors, as well as azo dyes, hexachloroethane, naphthalenes, anthracenes, phosphorus compounds, etc. as irritants.

Substances of natural origin could be used among the means of chemical destruction, and the use of products contaminated with botulinum toxin was revealed. Purification of the target protein using antibodies to various types of toxin made it possible to identify the characteristic peptides of botulinum toxin A. The growth of hogweed, borsa, hemlock, poisonous mushrooms and others on the territory also does not exclude the possibility of their use for terrorist purposes.

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Limitations. Analytical studies of samples do not apply to environmental objects, and the methodological possibilities of their interpretation are limited by the chemical nature of the analyte.

Conclusion

Thus, in the zone of a special military operation, the purposeful use of non-conventional means of chemical destruction was revealed, the form of application of which, including the mass-median characteristics of aerosol particles, determine the possibilities of achieving the target effect [10], difficulties in diagnosis and treatment. There is no doubt that the work on the selection and application of dual-use chemicals is coordinated and presupposes an uncompromising defeat of the military personnel and the population of the liberated territories of the Russian Federation from the point of view of international law.

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