Improvement of methods and means of measuring the content of toxicants in biological objects

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Abstract. The results of the development of a reference procedure for measuring the content of toxicants in biological objects are considered. To ensure traceability to the state primary standard, two types of certified reference materials of aqueous solutions of propanol-1 and propanol-2 have been developed, manufactured and approved. The mass spectra of narcotic psychotropic and psychoactive medicinal substances with fixed retention times were investigated. Mass spectra tables have been approval as standard reference data.

Introduction
The main objective of the research carried out by the chemical and toxicological laboratories of narcological dispensaries is the study of biological fluids of the human body (blood, urine, saliva) for the presence of alcohol and its surrogates, metabolites of narcotic drugs, psychotropic and other toxic substances and laboratory confirmation of alcoholic intoxication or intoxication as a result drug use, intoxication and the fact of consumption.

To obtain reliable results, chemical and toxicological laboratories should use measurement procedures with established metrological characteristics that meet mandatory metrological requirements. In accordance with the law "On Ensuring the Uniformity of Measurements", confirmation of compliance of measurement procedures (methods) with mandatory metrological requirements for measurements is carried out by certification of measurement procedures (methods).

Currently, methods for measuring the content of ethyl alcohol vapors in exhaled air have been developed, there are approved type measuring instruments designed for these purposes and means of their metrological support.

As for the measurements of the content of toxicants, narcotic and psychotropic substances in biological objects, such as blood, urine, serum and saliva, these are performed using analyzers that require individual calibration. Such devices include gas and liquid chromatographs (GC and LC), gas chromatograph - mass spectrometers (GC-MS). In this case, the content of toxicants can be determined using measurement procedures.

Measurements of the mass concentration of alcohol, the content of narcotic and psychotropic substances in biological objects are not included in the approved list of measurements related to the sphere of state regulation of ensuring the uniformity of measurements, although they are important when determination of the presence of alcohol or potent substances in the human body. Currently, only measurements of the mass concentration of ethanol vapors in the exhaled air are included in the mentioned list.
Development of a reference procedure and certified reference materials

To ensure the uniformity and reliability of measurements of the content of toxicants in biological objects, traceable to the existing state primary standards in the field of chemical and analytical measurements, a reference procedure for measuring the mass concentration of toxicants in biological objects was developed. The use of the reference procedure will make it possible to assess the correctness of measurement results obtained using other measurement methods of the same quantities.

The procedure is based on the method of gas chromatography with the introduction of samples by the method of equilibrium vapor phase with thermostating. The toxicant content was measured on a gas chromatograph with two flame ionization detectors and two capillary columns having different liquid phase polarity. An automatic vapor-phase sampler was used to enter samples [1].

As a result of the conducted studies, it was established that the accuracy indicators of the developed reference procedure for measuring the mass concentration of toxicants in biological objects were improved compared to the same indicators of existing methods of routine measurements of the same components in the same objects.

To ensure traceability to the state primary standard, certified reference materials of aqueous solutions of propanol-1 (GSO 11383-2019) and propanol-2 (GSO 11384-2019) were developed, manufactured and approved [2].

Propanol-1 and propanol-2 solutions were prepared in accordance with the developed preparation procedure. The sample preparation procedure was selected from three options obtained by varying weighed portions and capacities of volumetric flask.

Traceability to the state primary standard GET 208-2014 is confirmed by the use of reference standards for propanol-1 and propanol-2 to transfer a unit of mass fraction of these substances.

Investigation of mass spectra of topical narcotic drugs, psychotropic and psychoactive drugs

Unlike volatile toxicants, the problem of quantitative determination of the content of narcotic drugs, psychotropic and psychoactive drugs in biological objects has not yet been solved. The lack of a sufficient nomenclature of reference materials of narcotic drugs, psychotropic and psychoactive drugs, and the unavailability of certified samples containing these substances does not allow quantitative measurements of their content in biological objects (urine, blood, organs and tissues, hair and nails) during chemical and toxicological studies and forensic chemical examinations.

In this regard, it is currently advisable to develop and approve as standard reference data tables of mass spectra of narcotic drugs, psychotropic and psychoactive drugs with fixed retention times.

The mass spectra were studied for morphine hydrochloride, codeine, 6-acetylmorphine hydrochloride, amphetamine sulfate, 3,4-methylenedioxymethamphetamine hydrochloride (MDMA, ecstasy), cocaine hydrochloride, fentanyl tropicamide, baclofen, gabapentin, carbine clozapine, N-desmethylclozapine, doxylamine, haloperidol, amitriptyline.

Studies of the mass spectra of the listed substances were performed by gas chromatography-mass spectrometry (GC-MS) with electron ionization. Using a gas chromatograph makes possibility to solve the problem of separating a sample into components with automatic registration of their peak areas and retention times, and a mass spectrometer detects and identifies these components [3-5].

The method makes it possible to automatically identify target components by two analytical parameters: retention time and mass spectrum. Identification only by the mass spectrum makes it possible to determine a set of possible candidates of unknown compounds having similar mass spectra. Identification of the target component in this case is difficult [5].
Simultaneous mass spectral and retention time searches increase assay reliability and identify compounds with similar mass spectra.

Experimental studies were carried out at the Moscow Scientific and Practical Center for Narcology DZM using a 5977V GC / MSD gas chromatograph-mass spectrometer (registration number in FIF 65319-16), consisting of an Agilent 7890V gas chromatograph and an Agilent 5977V quadrupole mass-selective detector (Agilent Technologies, USA). Data processing was performed using Agilent MassHunter software (ver. B.06.00), Agilent Technologies, USA.

The advantage of quadrupole mass spectrometry is a larger dynamic range compared to alternative detectors, and the obtained mass spectra are the closest to the library ones [6].

The choice of such a GC-MS system, equipped with a chromatographic quartz capillary column HP-5ms (5-phenylmethylpolysiloxane) 30m * 0.25mm * 0.25μm, is due to the use of similar systems in chemical and toxicological laboratories for the determination of narcotic drugs, psychotropic and psychoactive drugs [6].

The results of the study of the mass spectra of narcotic drugs, psychotropic and psychoactive drugs with fixed retention times have been approval as tables of standard reference data.

The use of standard reference data tables will allow specialized laboratories that do not have the appropriate licenses to carry out qualitative measurements of these substances in order to identify them in biomaterial samples.

Conclusion
For the purposes of obtaining reliable results of chemical and toxicological laboratories and laboratory confirmation of alcohol intoxication and intoxication as a result of drug use, intoxication and the fact of consumption was developed and approval

- the reference procedure for measuring the mass concentration of toxicants (alcohols) in biological objects; certificate of the reference procedure No. 205-12/RA.RU.311787/2019, number in the Federal Information Fund FR.R1.31.2019.00006;
- certified reference materials (CRM):CRM of propanol-1 solution (GSO 11383-2019); CRM of propanol-2 solution (GSO 11384-2019);
- the tables of mass spectra of narcotic and psychotropic substances with fixed retention times were approval as standard reference dates.

References
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