INTRODUCTION

The abundance of acute overdoses with antihypertensive drugs and the similarity of their clinical implications necessitates the development of a screening method aimed at the exact detection of the toxicant. At the present state in bioanalytical investigations, for instance, in the laboratory diagnostics of acute overdose with these APDs, methods of chromatography, i.e., gas and high-performance liquid chromatography with mass spectrometry detection, are used most frequently [1–13].

The advantages of gas chromatography with mass spectrometry detection (GC–MS) are the high efficiency of separation of sample components, high sensitivity and selectivity, and a combination of screening with confirmation analysis within one method [14]. Substantial huge advantages of GC–MS for routine analysis are provided by the availability of vast libraries of mass spectra [15, 16]. The commonly accepted standard of registration in GC–MS is ionization by electrons with an energy of 70 eV, which ensures a higher stability of mass spectra recorded by various instruments in different places. The disadvantages of GC–MS are restrictions due to the nature of analytes: the decomposition of thermally labile substances in the injection port of the chromatograph at high temperatures, and the necessity of the derivatization of polar molecules [17].

Method of high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) has no such drawbacks; now the majority of analysts give their preferences to this method because of its versatility. It is irreplaceable in the search for a specific toxicant, in the determination of non-volatile and polar compounds, and also as an universal and sensitive method for the quantitative determination of drugs in biological fluids [18, 19]. However, its use for screening is limited because of the rapid contamination and, therefore, reduction of detector sensitivity in routine analyses [20]. The milder ionization conditions compared to GC–MS conditions result in less informative mass spectra in HPLC–MS/MS, which may lead to false positive conclusions about the presence of toxicants [21]. The conditions of analysis by HPLC–MS/MS are not standardized because of the use of many different types of mass spectrometry detectors and ion sources; therefore, spectral libraries are not so abundant as for GC–MS.

The disadvantages of GC–MS become especially visible in the determination of antihypertensive drugs. These compounds are insufficiently thermally stable and decompose in the chromatograph to a greater extent, which deteriorates sensitivity and reproducibility in their determination by GC–MS methods without derivatization. Many analytical methods for determining antihypertensive drugs by GC–MS with derivatization were described, in which this problem was solved [13, 14, 17]. The step of derivatization complicates sample preparation and elongates the analysis, while in the laboratory diagnostics of acute states, in contrast, it is desirable to shorten the time of
injection as much as possible. In case of overdose with antihypertensive drugs, the concentrations of toxicants in blood significantly exceed the therapeutic level, so the use of GC–MS without derivatization is acceptable. Therefore, the detection of acute overdoses by GC–MS without derivatization has still remained an task of current importance [22–24].

The aim of this work was the development of rapid screening method for detecting acute overdoses with antihypertensive drugs by GC–MS without derivatization.

**EXPERIMENTAL**

**Analysis by GC–MS.** The investigation was accomplished on a Thermo Trace GC Ultra gas chromatograph equipped with a DSQ II mass spectrometry detector. A TR-5MS column, column length 30 m, inner diameter 0.25 mm, stationary phase film thickness 0.25 μm, was used. The stationary phase consisted of 5% diphenyl and 95% dimethylpolysiloxane. The carrier gas was helium. The temperature program was as follows: isothermal period at 50°C for 0.5 min, heating at a rate of 100 K/min to 190°C, isothermal period at 190°C for 1 min, heating at a rate of 10 K/min to 280°C, and isothermal period at 280°C for 13 min. The temperature of the injector was 220°C; of interface, 280°C; and of ion source, 200°C. Registration was performed in the total ion current mode in the range m/z 45-650 using ionization by electron impact with an energy of 70 eV. The registration of signal was started 4.5 minutes after the sample was injected. The injected volume was 2 μL. The samples were injected into chromatograph in the splitless mode.

The data analysis was performed using NIST [15] and PMW [16] mass spectral databases.

**Reference solutions.** At the step of method development, standard solutions of propranolol, bisoprolol, metoprolol, atenolol, nifedipine, and enalapril with the concentration of the target substance 10 μg/mL were prepared from respective APDs. To each solution diphenylamine (DPA) was added in equal concentration, 10 μg/mL. The solvent was ethyl acetate.

**Preparation of urine samples to GC–MS analysis.** We collected urine samples taken from patients hospitalized with implications of the acute overdose with antihypertensive drugs. Sample pretreatment was accomplished by liquid–liquid extraction using conventional extraction systems Toxi Tube A (Agilent Technologies) for neutral and weakly basic substances, and Toxi Tube B (Agilent Technologies) for weakly acidic and neutral substances. Toxi Tube A contained 2.3 mL of a mixture heptane–isopropanol–1,2-dichloethane–dichloromethane as an extractant (77 : 38 : 58 : 58), NaCl as a saline agent, and a carbonate buffer. Toxi Tube B consisted of 2.3 mL of a mixture heptane–dichloromethane (105 : 125), ZnCl₂ as a saline agent, and an acidifying agent. The procedures of sample pretreatment were identical for both systems.

A 3-mL portion of urine was charged into a Toxi Tube followed by 30 μL of an internal standard, DPA, concentration 20 μg/mL. The concentration of DPA in a sample was 200 ng/mL.

Extraction was accomplished in a shaker at a frequency of 100 rpm for 3 min. The phases were separated by centrifugation for 5 min at 3200 rpm. The organic phase, the upper one, was collected and evaporated. The dry residue was reconstituted with 200 μL of ethyl acetate or methanol. A 2-μL portion of the extract prepared was injected into the chromatograph.

A Toxi Tube A was utilized for screening with no specific focus or in order to confirm an overdose with APDs of weakly basic or neutral character, i.e., propranolol, atenolol, metoprolol, bisoprolol, and nifedipine. For the extraction of enalapril, which possesses weakly acidic properties, a Toxi Tube B was recommended. However, as enalapril can be extracted from urine in the weakly alkaline pH region and for the development of a common method of sample pretreatment in screening with no focus, we chose extraction with a Toxi Tube A system.

To evaluate the sensitivity of the method, we prepared model mixtures based on urine initially containing no analytes with spikes of APDs under consideration in concentrations of 30, 50, 100, 500, 1000, and 5000 ng/mL. The sample pretreatment of model mixtures was accomplished as described above.

**Generation of β-adrenoblocker artifacts.** In these experiments, 50 μL of a standard solution of a β-adrenoblocker with the concentration 100 μg/mL was charged into a screw-capped tube followed by 2.5 mL of purified water and 0.5 mL of a 40% solution of formaldehyde. Then the mixture was heated on a sand bath for 15 min and subjected to extraction with 3 mL of ethyl acetate on a shaker at 100 rpm for 3 min. Then the test tubes were centrifuged for 5 min at 3200 rpm. The organic phase, the upper layer, was collected and evaporated. The dry residue was redissolved in 200 μL of ethyl acetate. A 2-μL portion of the extract prepared was injected into the chromatograph.

**RESULTS AND DISCUSSION**

In the work with standard solutions of antihypertensive drugs, we optimized chromatography conditions and could reach the full resolution of all APDs in question. As was found, β-adrenoblockers, i.e., atenolol, bisoprolol, metoprolol, and propranolol, under these conditions form artifacts; their peaks in chromatograms are eluted later than the peaks of respective titular substances by approximately 0.75 min. The shapes of artifact peaks are better, they are narrower and higher in