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ARTICLES

Chromatographic Study of Expert and Biological Samples Containing Desomorphine

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Abstract—Expert and biological samples containing desomorphine and concomitant compounds were studied by gas chromatography, chromatography–mass spectrometry, and high-performance liquid chromatography. Some synthetic analogues of desomorphine were identified, and their chromatographic parameters and mass spectra were described. Techniques for the extraction and study of desomorphine and didehydrodesomorphine on a Milichrom A-02 liquid chromatograph were outlined. This information is important for experts in analytical toxicology and criminalistics.

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In recent years, a drastic increase was observed in the number of cases of abuse of synthetic narcotics produced by handicraft techniques from codeine-containing medicines. Tablets available at cost supplied in drugstores without prescription, such as Codterpin, Codelac, Sedal-M, etc., are used for the synthesis. The amount of codeine in these medicines is 8–10 mg. Desomorphine (5a-17-methyl-4,5-epoxymorphinan-3-ol) is obtained from codeine-containing tablets with the use of iodine and phosphorus. In terms of the strength of impact on the body, this compound significantly surpasses codeine.

Cases of withdrawal symptoms of synthetic narcotics of this type occurred in many regions of Russia. Desomorphine is included in the "List of Narcotics, Psychotropic Substances, and Precursors Thereof, Subject to Control in the Russian Federation" [1]. Therefore, the comprehensive study of this compound in expert materials and biological samples is an important problem.

Expert studies of withdrawn samples containing desomorphine involve difficulties. Primarily, these difficulties are due to the lack of information about features of the chemical analysis of this compound and the absence of standard reference materials. Descriptions of possible byproducts in the chain of transformations from codeine to desomorphine at different conditions of their synthesis could not be found in the literature. Practice demonstrated that, in different regions, desomorphine is synthesized under different conditions and by different procedures. Hence, the resulting samples differ from each other in the concentration of desomorphine and in the composition of concomitant reaction products.

Therefore, the aim of this work was (i) to study the component composition of desomorphine-containing samples by gas chromatography with a mass-selective detector (GC-MS) and (ii) to develop a procedure for the determination of desomorphine on a Milichrom A-02 liquid chromatograph in expert materials and biological fluids of the human body.

This information is necessary for experts in criminalistics and analytical toxicology.

EXPERIMENTAL

Instruments and procedure. The GC-MS analysis was performed on an Agilent 6850 chromatograph with an Agilent 5973 mass-selective detector (Agilent Technologies). The conditions of chromatography were as follows.

Mode 1. An HP-SM5 column with a diameter of 0.35 mm and a length of 30 mm; the thickness of the phase film 0.33 mm. Carrier gas, helium; flow rate of the carrier gas in the column, 1.5 mL/min. Splitless mode. Injector temperature, 270°C; column temperature programming: first step, 50°C for 1 min, next with a rate of 99 K/min up to 100°C; second step, 100°C for 1 min, next with a rate of 15 K/min up to 280°C; third step, 280°C for 20 min; MSD interface temperature,

280°C. Electron impact ionization with an electron energy of 400 eV. MSD operation mode: full scan of ions from 40 to 450 amu (SCAN mode). Data processing was carried out on a chemical station Enhanced Head Station G-1701 DA Version D.00.00.38. The obtained mass spectra were compared with library mass spectra (NIST-98 Mass Spectral Library provided with the processing program of the instrument) and processed with the program Amdis version 2.1. The GC-MS analysis was performed by the retention time-locking method reported in [2].

Mode 2 differs from the former in column temperature programming: first step, 100°C for 1 min; next with a rate of 35 K/min up to 300°C for 10 min.

Liquid chromatography was performed on a Milichrom A-02 chromatograph with a UV detector (ZAO EcoNova, Novosibirsk, Russia). Conditions of chromatography: Protosil-120-5-C18 AQ column; mobile phase A, (4 M $LiClO_4$–0.1 M $HClO_4$) : H_2O (95 : 5); mobile phase B, acetonitrile of an HPLC grade; gradient from 5 to 100% mobile phase B in a volume of 4000 μL, next 100% mobile phase B in a volume up to 4500 μL; flow rate of the eluent, 100 μL/min; column temperature, 40°C. Detection was performed in the multiwave mode at 210, 220, 230, 240, 250, 260, 280, and 300 nm. The volume of the injected sample was 4 μL.

Compounds were identified by the retention volumes and spectral ratios according to methodological reconditions from compendium [3]. Spectral ratios were calculated by the best purity method.

For thin-layer chromatography (TLC), we used Sorbfil PTSKh-AF-UF plates. As reference samples, we used ethanolic solutions of morphine, codeine, and diacetylmorphine. Chromatography was performed in a solvent system benzene–ethanol–diethylamine (9 : 1 : 1). The solvent front path was 90 mm. After the end of chromatography, the plates were dried, examined under UV rays (OLD-41 lamp) marking fluorescence absorption zones, and then developed with the Marki reagent (solution of formaldehyde in concentrated H_2SO_4). For the extraction of compounds, four spots were applied to a plate, one of the spots was developed with the Marki reagent, and the other zones were eluted at the level of the developed compounds with a chloroform–isopropanol mixture (9 : 1).

Samples and their preparation for the analysis. We studied expert-forensic and biological samples. The expert-forensic samples were washouts from cotton-wool tampons (through which persons consuming narcotics filtered synthesis products before intravenous introduction), washouts from used syringes, and residues of liquids in syringes. Biological fluids were commonly urine taken from persons consuming desomorphine from different regions, in particular, the Kemerovo and Lipetsk regions.

Expert samples were washed with water acidified with 0.1 M HCl to pH 2. The resulting washouts were centrifuged, alkalified with sodium hydrogen carbonate to pH 8, and extracted with a chloroform–isopropanol mixture (9 : 1). The organic phase was evaporated, the dry residue was dissolved in 200 μL of a chloroform–isopropanol mixture (9 : 1), and the resulting extracts were studied by TLC and GC-MS under the conditions described above.

Urine for the determination of narcotics and medicines was taken as described in [4]. Three milliliters of urine was alkalified with sodium hydrogen carbonate to pH 8 and extracted with a chloroform–isopropanol mixture (9 : 1) The organic phase was evaporated, the dry residue was dissolved in 200 μL of a chloroform–isopropanol mixture (9 : 1), and the resulting extracts were also studied by thin-layer chromatography and GC-MS.

The hydrolysis of urine samples and the isolation of narcotics of the opium group were performed as described in [5].

The derivatization of samples was performed as follows: (i) BSTFA (100 μL) was added to the extract evaporated to dryness, and the mixture was kept for 30 min at 55°C to obtain trimethylsilyl derivatives. The sample was evaporated to dryness, and the residue was dissolved in 50 μL of ethyl acetate. (ii) MBTFA or TFAA (100 μL) was added to the extract evaporated to dryness, and the mixture was kept for 1 h at 70°C to obtain trifluoroacetyl derivatives. The sample was evaporated to dryness, and the residue was dissolved in 50 μL of ethyl acetate.

RESULTS AND DISCUSSION

For the determination of the component composition of desomorphine-containing samples and sample extracts, we used GC-MS; all compounds that were expected to be synthetic analogues of codeine were identified, and their relative concentrations were determined. The results are presented in Tables 1 and 2.

Table 1 presents the main codeine derivatives identified by GC-MS that were found in expert samples and samples of urine. In the studied samples, caffeine, dimedrol, analgin and its decomposition products, and phenobarbital were also found. For nearly all of the studied samples except those listed above, the chromatograms exhibit the peak of a compound (its mass spectrum is presented in Fig. 1) that was provisionally called compound 1.

Table 2 presents the codeine derivatives found in the studied samples. The concentrations of the identified components were determined by the internal normalization method, in which all peaks in the chromatogram were taken as 100%.

As seen in Table 1, the studied substances in different samples substantially differed in composition and mutual ratios of components. This is due to the specific features of the synthesis, its steps, the technique of the preparation of the narcotic for use, and the purification

Table 1. Compounds identified as synthetic analogues of codeine, which were found in extracts from expert samples

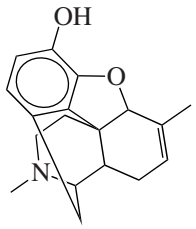
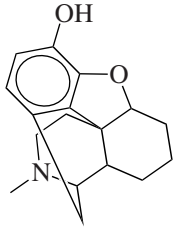
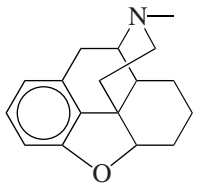
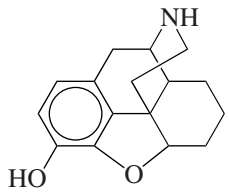
Chemical name	Number	Structural formula	Chemical name	Number	Structural formula
Methyldesomorphine	16008-36-9		Desomorphine	427-00-9	
Characteristic ions			Characteristic ions		
283 160 42	268 226		271 270 214	148 272	
Dihydromorphine-3,6-dideoxy-	69663-72-5		Morphinan-4,5-epoxy-3-ol	29096-51-3	
Characteristic ions			Characteristic ions		
255 42 254	256 198		257 214 45	256 258	

Table 2. Concentrations of the identified compounds in expert samples and urine

Sample	Compound	Concentration, %	Sample	Compound	Concentration, %
Expert sample no. 1	Dihydromorphine-3,6-dideoxy-	5.19	Expert sample no. 5	Dihydromorphine-3,6-dideoxy-	15.14
	Methyldesomorphine	33.79		Morphinan-4,5-epoxy-3-ol	62.75
	Desomorphine	29.58		Compound 1	2.28
	Compound 1	29.67		Desomorphine	19.12
	Codeine	1.759		Codeine	0.710
Expert sample no. 2	Desomorphine	8.130	Urine extract no. 1	Desomorphine	75.62
	Compound 1	76.215		Dihydromorphine-3,6-dideoxy-	24.37
	Codeine	15.655			
Expert sample no. 3	Methyldesomorphine	20.84	Urine extract no. 3	Desomorphine	traces*
	Desomorphine	11.83			
	Compound 1	65.23		Compound 1	55.94
	Codeine	2.09		Codeine	44.05

* In urine extract no. 3, desomorphine was identified only by special ions.

of products. In particular, in sample no. 2, the concentration of compound 1 is significantly larger than the concentrations of desomorphine and codeine. The concentration of desomorphine in the extracts of urine samples varies from 70–80% to trace amounts.

Along with desomorphine, its derivatives in different concentrations were also found in studies of the urine samples. This indicates that a part of these components that arrive at the body together with desomorphine are excreted in an unchanged form. Desomorphine and other conversion products of codeine are identified in the urine samples either after acidic hydrolysis or without hydrolysis. In chromatograms of an extract of urine containing large amounts of analgin, the analgin peak masks the desomorphine peak. In these cases, it is preferable to identify desomorphine in hydrochloric acid hydrolyzates of samples, where the interfering effect of analgin is decreased.

Because compound 1 occurs in many samples containing desomorphine, we assumed that this compound is its derivative. Figure 2 presents a chromatogram of a sample extract in mode 2, where the peaks of the compounds with retention times of 7.49 and 7.53 min were identified as desomorphine and compound 1, respectively.

The structure of compound 1 was identified by the structure–spectrum correlation method on the basis of

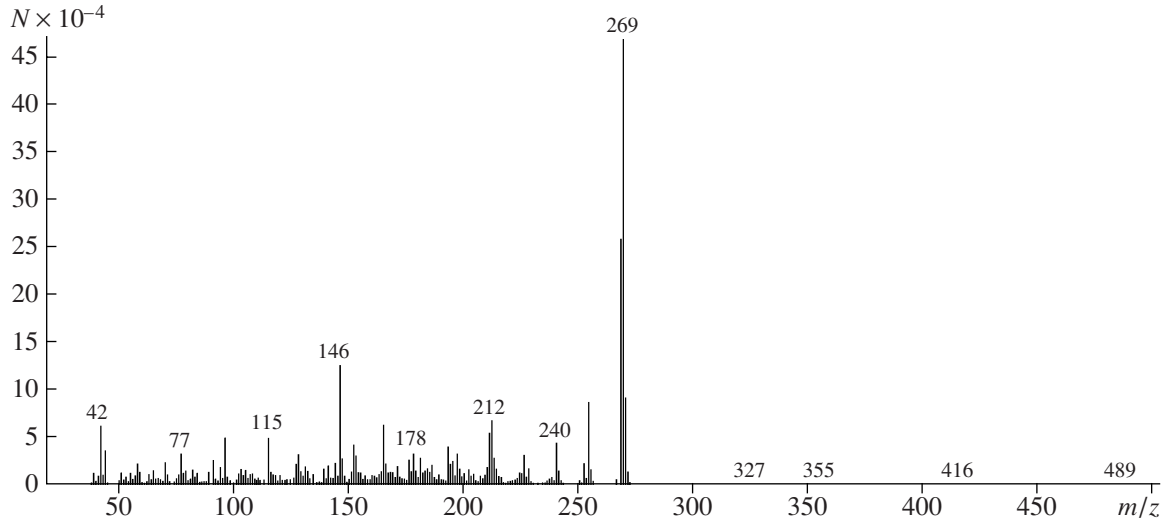


Fig. 1. Mass spectrum of compound 1 (retention time, 14.240 min).

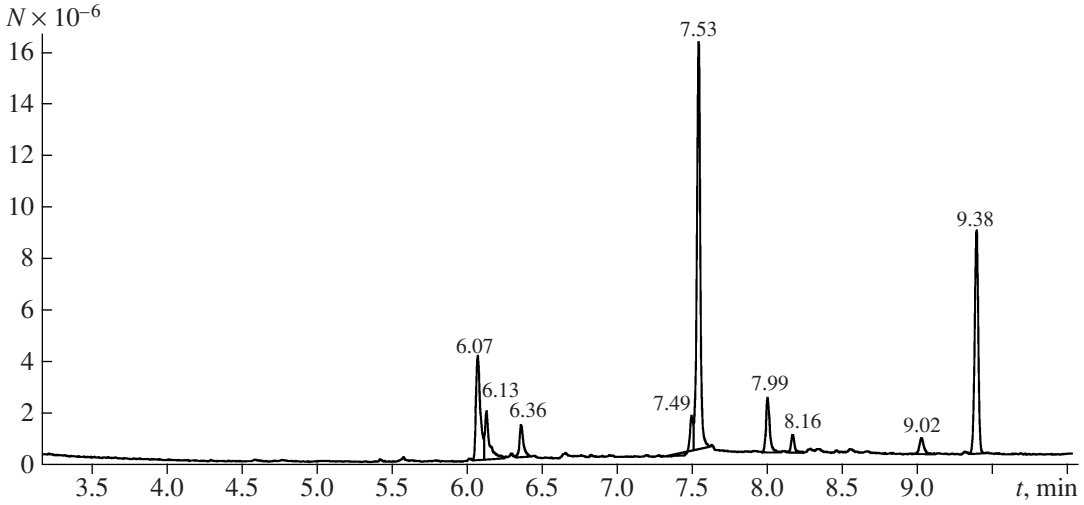


Fig. 2. Chromatogram of the extract of a sample containing desomorphine.

the known mass-spectrometric destructions of desomorphine and its analogues.

As is known, the molecular ion m/z = 271 is the main ion in the mass spectrum of desomorphine. The ion m/z = 256 $[M-15]^+$ corresponds to the loss of the methyl group, the ion m/z = 254 $[M-17]^+$ is characteristic of the loss of the hydroxy group, m/z = 242 $[M-29]^+$ is possibly the loss of the C_2H_5 species with the cleavage of one of the unsaturated rings of the molecule, m/z = 228 $[M-43]^+$ is the loss of the C_2H_6N species, and m/z = 214 $[M-57]^+$ is the loss of the C_4H_9 fragment, which can correspond to the loss of the methyl group and the C_3H_7 species with the cleavage of two bonds at the 5.6 and 8.14 positions of the desomorphine molecule.

If we assume that the main direction of the fragmentation of compound 1 is the same as for desomorphine, the mass spectrum of this compound can be treated as follows (see Fig. 1): m/z = 269 is the molecular ion that differs from desomorphine by 2 amu; this allows the assumption that the molecule of compound 1 has one double bond more than desomorphine; m/z = 254 $[M-15]^+$ corresponds to the loss of the methyl group, m/z = 252 $[M-17]^+$ corresponds to the loss of the hydroxy group, m/z = 240 $[M-29]^+$ corresponds to the possible loss of the C_2H_5 species with the cleavage of one of the unsaturated rings of the molecule, m/z = 226 $[M-43]^+$ corresponds to the loss of the C_2H_6N species, and m/z = 212 $[M-57]^+$ corresponds to the loss of the C_4H_9 fragment, which can correspond to the loss of the methyl

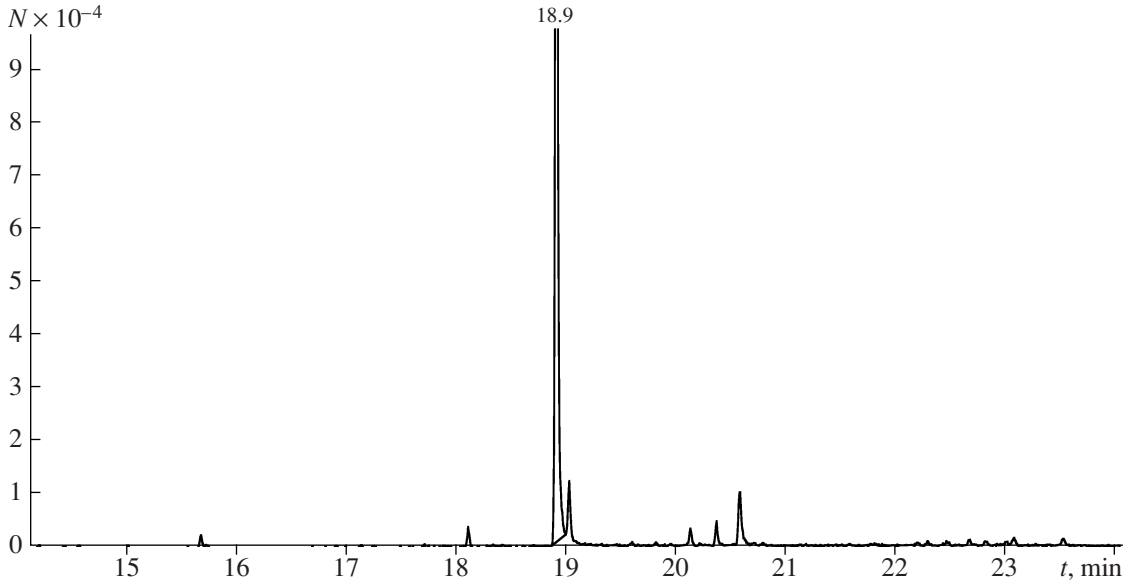


Fig. 3. Chromatogram of the trimethylsilyl derivative of desomorphine.

group and C_3H_7 with the cleavage of two bonds at the 5.6 and 8.14 positions.

Note that the studied compound has a destruction character similar to that of desomorphine. In fragmentation, the destruction of two unsaturated rings is observed: one of them involves nitrogen and, in the other, the cleavage of two opposite bonds is observed, possibly, at the 5.6 and 8.14 positions. Because all fragments of the studied compound differ from the fragments in the mass spectrum of desomorphine by 2 amu, it is expected that the double bond is localized in the saturated ring that is not destructed in the fragmentation. The identified compound can be represented as didehydrodesomorphine.

Because the derivatization of compounds is use in the GC-MS analysis of narcotics in forensic studies and studies of biomaterials, the study trifluoroacetyl and trimethylsilyl derivatives of desomorphine and concomitant compounds is of great practical interest. However, we failed to find information on these derivatives in the available literature. Therefore, the preparation of the trifluoroacetyl derivative of identified didehydrodesomorphine and the study of its mass spectrum can, in our opinion, confirm the structure of the studied compound.

In the BSTFA derivatization of desomorphine in an urine extract, we obtained a peak with a retention time of 18.897 min; the chromatogram (in mode *1*) and the mass spectrum are presented in Figs. 3 and 4.

The molecular mass of the trimethylsilyl derivative of desomorphine is 343 amu; consequently, the molecular ion corresponds to $m/z = 343$, the ion $m/z = 328$ $[M\text{-}15]^+$ corresponds to the loss of the methyl group, the ion $m/z = 314$ $[M\text{-}29]^+$ reflects the possible loss of the C_2H_5 species with the cleavage of one of the unsaturated rings, $m/z = 300$ $[M\text{-}43]^+$ reflects the loss of the C_2H_3N species, $m/z = 286$ $[M\text{-}57]^+$ is the loss of the C_4H_9 fragment, which can correspond to the loss of the methyl group and C_3H_7 with the cleavage of two bonds at the 5.6 and 8.14 positions of the saturated ring, and the ion $m/z = 271$ $[M\text{-}72]^+$ can correspond to the loss of the trimethylsilyl group.

By the character of destruction, this compound can be identified as the mono(trimethylsilyl) derivative of desomorphine. Generally, the character of the destruction is identical to the destruction of desomorphine. The ion corresponding to the detachment of the hydroxy group $[M\text{-}17]^+$ is absent, because the derivatization occurs at the hydroxy group, and the ion $[M\text{-}72]^+$ corresponding to the loss of the trimethylsilyl group is observed.

Figures 5 and 6 present the chromatogram of the trifluoroacetyl derivative of desomorphine and its mass spectrum. In chromatography in mode 1, the peak of the trifluoroacetyl derivative of desomorphine had a retention time of 11.85 min.

The molecular ion $m/z = 367$ is the main ion in the mass spectrum. The ion $m/z = 352$ $[M\text{-}15]^+$ corresponds to the loss of the methyl group, the ion $m/z = 338$ $[M\text{-}29]^+$ is possibly the loss of the C_2H_5 species with the cleavage of one of the unsaturated rings of the molecule, $m/z = 324$ $[M\text{-}43]^+$ is the loss of the C_2H_6N species, $m/z = 310$ $[M\text{-}57]^+$ is the loss of the C_4H_9 fragment, which can correspond to the loss of the methyl group and the C_3H_7 species with the cleavage of two bonds at the 5.6 and 8.14 positions, and $m/z = 270$ $[M\text{-}97]^+$ is the loss of the trifluoroacetyl group. Similarly to the mono(trimethylsilyl) derivative of desomorphine, the ion corresponding to the detachment of the hydroxy

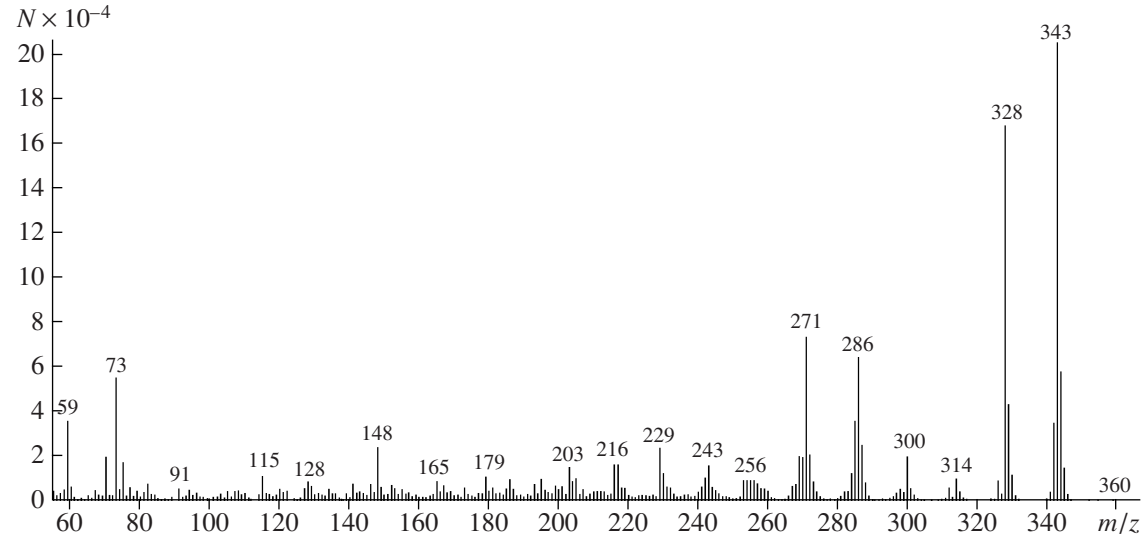


Fig. 4. Mass spectrum of the trimethylsilyl derivative of desomorphine (retention time, 18.897 min).

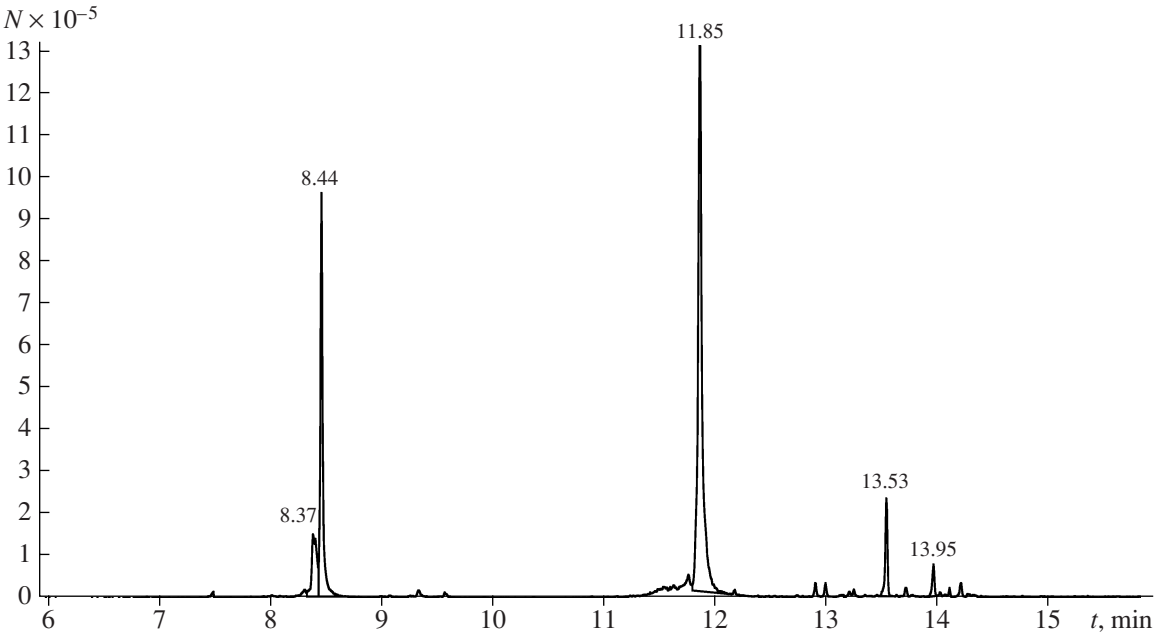


Fig. 5. Chromatogram of the trifluoroacetyl derivative of desomorphine.

group $[M-17]^+$ is absent, because the derivatization occurs at the hydroxy group, and the ion $[M-97]^+$ corresponding to the loss of the trifluoroacetyl group is observed. By the character of destruction, this compound can be identified as the mono(trifluoroacetyl) derivative of desomorphine.

To confirm the structure of the identified didehydrodesomorphine, we studied the product of its MBTFA derivatization. The chromatogram and the spectrogram of the compound are presented in Figs. 7 and 8. Chromatography was performed in mode 2; the peak of the studied compound has a retention time of 6.98 min.

The trifluoroacetyl derivative of didehydrodesomorphine must have the molecular ion $m/z = 365$; in addition, the spectrogram exhibits peaks of ions $m/z = 350$ $[M-15]^+$ corresponds to the loss of the methyl group, $m/z = 336$ $[M-29]^+$ possibly corresponding to the loss of the C_2H_5 species with the cleavage of one of the unsaturated rings of the molecule, $m/z = 322$ $[M-43]^+$ corresponding to the loss of the C_2H_6N species, and $m/z =$

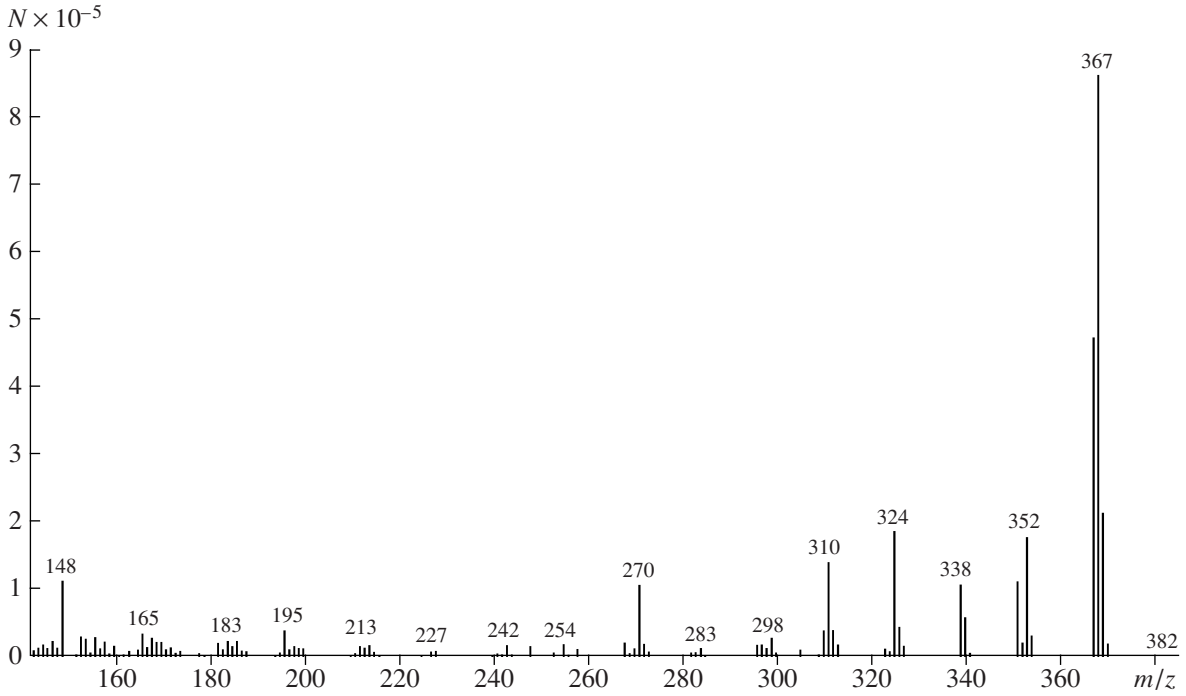


Fig. 6. Mass spectrum of the trifluoroacetyl derivative of desomorphine (retention time, 11.854 min).

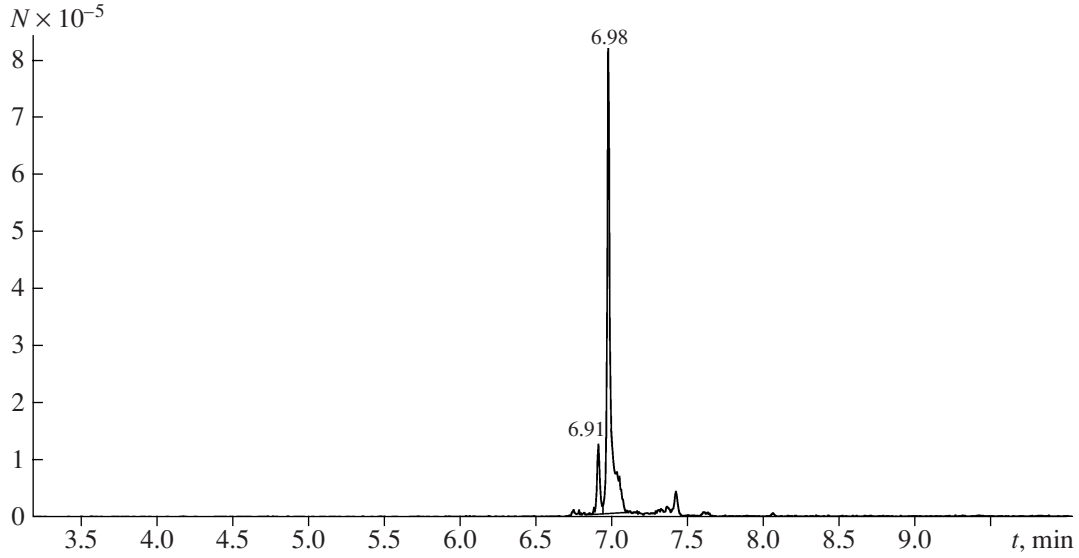


Fig. 7. Chromatogram of the trifluoroacetyl derivative of didehydrodesomorphine.

268 $[M-97]^+$ corresponding to the loss of the trifluoroacetyl group.

Similarly to other trifluoroacetyl derivatives, the ion corresponding to the detachment of the hydroxy group $[M-17]^+$ is absent, because the derivatization occurs at the hydroxy group, and the ion $[M-97]^+$ corresponding to the loss of the trifluoroacetyl group is observed. By the character of destruction, this compound can be identified as the mono(trifluoroacetyl) derivative of didehydrodesomorphine.

Thus, desomorphine was found in GC-MS studies of different expert samples. For the first time, didehydrodesomorphine, the mono(trifluoroacetyl) derivative of didehydrodesomorphine, the mono(trifluoroacetyl) derivative of desomorphine, and the mono(trimethylsilyl) derivative of desomorphine were identified by GC-MS.

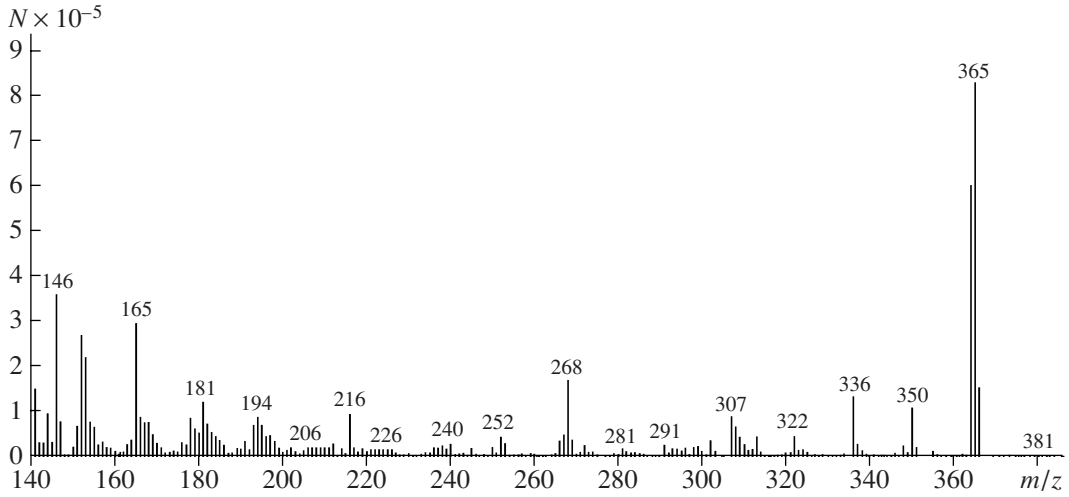


Fig. 8. Mass spectrum of the trifluoroacetyl derivative of didehydrodesomorphine (retention time, 6.973 min).

Before studying desomorphine and didehydrodesomorphine by HPLC, it was necessary to extract these compounds; previously, they were purified by thin-layer chromatography. The solvent system benzene–ethanol–diethylamine (9 : 1 : 1) was used as most frequently used in the analysis of narcotics of the opium group. In the thin-layer chromatography of an expert sample containing desomorphine, two spots were observed on the plate of development with the Marki reagent: a hardly noticeable blue–violet spot at a level of codeine (spot 1) and a blue–violet spot in the zone between codeine and diacetylmorphine, the zone corresponding to desomorphine (spot 2).

Next, compounds from the TLC plate (at the level of the developed zones) were eluted with a chloroform–isopropanol mixture (9 : 1). A part of the obtained extracts were studied by GC-MS; the results are presented in Table 3. The concentrations of identified components were determined by the internal normalization method, in which all identified peaks were taken to be 100%.

Thus, in the expert sample, the compounds developed by the Marki reagent were found in two zones. In the first zone at the level of codeine, didehydrodesomorphine and codeine were tentatively found in approximately equal amounts. In the second zone at the level of desomorphine, didehydrodesomorphine and desomorphine were found (also tentatively), and the amount of desomorphine was three times smaller than the amount of didehydrodesomorphine. Analgin was also identified in this zone. Thus, under the given conditions, the separation of desomorphine from other synthetic analogues on the TLC plates is incomplete. The solvent system benzene–ethanol–diethylamine (9 : 1 : 1), which is used in the analysis of narcotics, is insufficiently informative; therefore, other methods must be used to confirm the presence of desomorphine in the studied samples.

For this purpose, purified samples were studied on a Milichrom A-02 liquid chromatograph in the mode described in [3]. The extracts after TLC purification were back extracted with 200 μL of a mixture (95 : 5) of 4 M $LiClO_4$–0.1 M $HClO_4$: H_2O and studied on the chromatograph.

Figures 9 and 10 and Table 4 present the results of the chromatographic separation of the purified extracts. As seen in the table, the peak of analgin and two unresolved peaks at the level of codeine were found in the extract from spot 1. Taking into account the spectral ratios, chromatographic peak areas, and the results of GC-MS and TLC studies, peaks 5 and 6 can be identified as didehydrodesomorphine and codeine, respectively. Two peaks with an area ratio of approximately 1 : 3 were found in the extract from spot 2. Taking into account the spectral ratios, chromatographic peak

Table 3. Concentrations of the identified compounds in samples purified by thin-layer chromatography

Sample	Compound	Concentration, %	Sample	Compound	Concentration, %
Spot 1 at the level of codeine	Didehydrodesomorphine	56.76	Spot 2 at the level of desomorphine	Didehydrodesomorphine	86.23
	Desomorphine	1.197			
	Codeine	42.04		Desomorphine	13.77

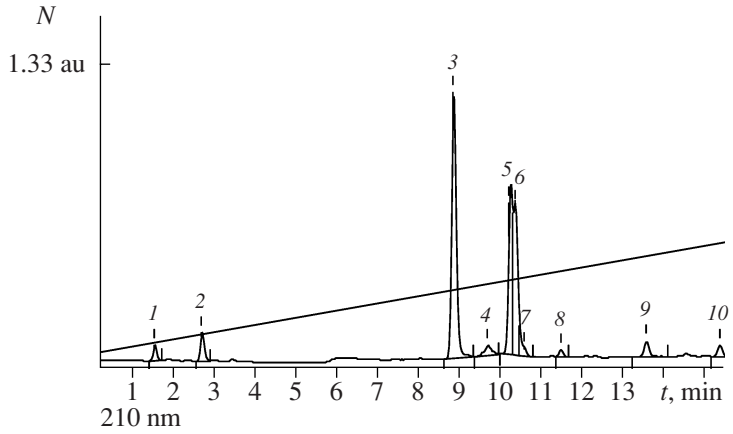


Fig. 9. Chromatograms of spot 1 at the level of codeine.

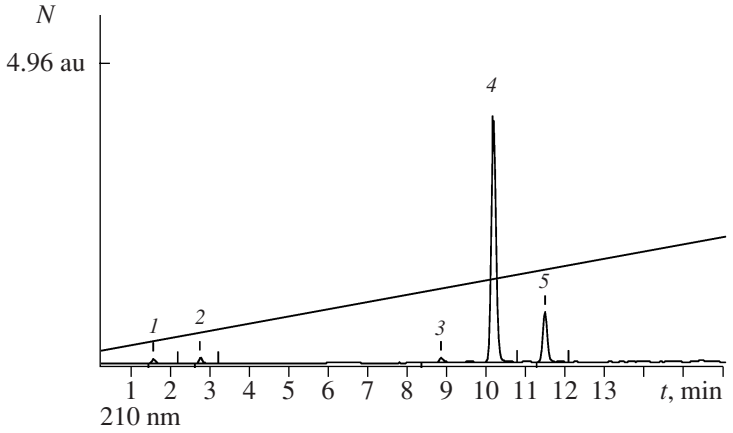


Fig. 10. Chromatograms of spot 2 at the level of desomorphine.

areas, and the results of GC-MS and TLC studies, the peak with a retention time of 10.15–10.18 min can be identified as didehydrodesomorphine and the peak with the retention time of 11.46 min can be identified as desomorphine.

In conclusion, we cam summarize the results of this study.

(i) In the GC-MS analysis of different expert samples containing desomorphine, four components were found and identified (by mass spectra) as synthetic ana-

Table 4. Results of the processing the chromatographic spectra of spots at the level of codeine and desomorphine

Peak number	Retention volume, μL	Peak area, arb. units	Spectral ratios, nm							Compound
			220	230	240	250	260	280	300	
Spot 1 at the level of codeine										
3	881.96	15.304	0.708	0.615	0.695	0.710	0.787	0.349	0.016	Analgin
5	1018.22	8.293	0.352	0.191	0.111	0.036	0.017	0.050	0.002	Didehydrodesomorphine
6	1033.74	8.567	0.674	0.241	0.201	0.128	0.042	0.072	0.008	Codeine
Spot 2 at the level of desomorphine										
4	1015.34	55.027	0.363	0.197	0.115	0.039	0.017	0.050	0.002	Didehydrodesomorphine
5	1146.40	10.893	0.251	0.164	0.081	–0.001	0.005	0.040	0.000	Desomorphine

logues of desomorphine. The fraction of desomorphine varied from traces to 70–80% of the sum of all peaks. Consequently, in the determination of these compounds, it can be assumed that the studied sample is a product of the synthesis of desomorphine. The trifluoroacetyl and trimethylsilyl analogues of desomorphine were obtained and studied. Didehydrodesomorphine was identified in the samples; the mass spectra of this compound and its trifluoroacetyl derivative were observed.

(ii) Expert samples of urine containing desomorphine were studied. It was demonstrated that desomorphine can be either a major component or a trace impurity. Some synthetic analogues of desomorphine were identified in the urine samples. This indicates that a part of these compounds is excreted with urine in the unchanged form. Desomorphine is found in urine both on alkaline extraction and after hydrochloric acid hydrolysis.

(iii) By TLC purification, desomorphine was extracted and studied for the first time on a Milichrom A-02 liquid chromatograph. The retention times and spectral ratios of didehydrodesomorphine and desomorphine were determined under the conditions of the DB-2003 mode of the chromatograph.

Thus, this work involved the complex study of expert samples and biological fluids containing desomorphine, improved the quality of expertise, and called attention to the thoroughness of studies related to the illicit trafficking of narcotics.

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