European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

Comprehensive Method For Forensic Study Of Pregabalin And Tramadol Trace Amounts Using Gas-Liquid Chromatography With Mass Spectrometric Detector And Ir-Spectrophotometry

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ABSTRACT
Research objective: to study the possibility of using the method of chromato-mass-spectrometry combined with IR-spectrophotometry in the forensic chemical analysis of trace amounts of unknown poisonous and superpotent substances.
Material and methods. For the forensic study a material evidence – an empty glass and a used empty disposable syringe were provided by the judicial and investigation authorities, taken from the crime scene. The following tasks were set before the experts: to find traces of any substances in the glass and syringe and reveal the origin of the poisonous and superpotent substances; determine the name of the substances.
Results. During the conducted analysis using gas-liquid chromatography with the mass spectrometric detector and IR spectrophotometry, the complex method was developed for the forensic chemical examination of pregabalin and tramadol trace amounts on the object-carriers. The research established the analysis parameters such as retention time, molecular and fragmentation ions, their intensity, individual fragmentation of pregabalin and tramadol. IR spectra were obtained with characteristic passbands of functional groups related to pregabalin and tramadol structures.
Conclusion. The research proved that application of the complex methods, which have high sensitivity, speed and simplicity in use, affords to quickly and accurately identify unknown substances in the composition of micro-objects.

Key-words: forensic study, pregabalin, tramadol, gas-liquid chromatography, IR spectrophotometry, trace amount.

1. INTRODUCTION

When investigation and judicial examination of criminal cases on fatal poisoning, an increasing evidential significance is given to the micro-objects found at a crime scene. Object-carriers in this case are various items, syringes, ampoules, tubes, vials, glasses, etc., associated with the crime event. In these cases, the investigation and judicial authorities are interested in such questions as: are there any traces of a poisonous substance in the material evidence; if there are any, which one; determine its name; is it included in the list of narcotic drugs, psychotropic substances or precursors, and etc.

Forensic chemical examination of poisonous and superpotent substance trace amounts has a number of distinctive features associated with the improvement of the methods. This is due to the small quantity of substances, multiplicity of their forms, and the variety of objects-carriers. Successful solution of such problems depends on the application of more adequate and accurate methods.

Formerly, for the detection of traces of narcotic and psychotropic substances, precursors, and some poisonous and superpotent drugs on the objects-carriers, we had developed an analysis method using a gas chromatography-mass spectrometry, as well as HPLC with a mass spectrometric detector [1, 2, 3, 4].

Research Objective

The aim of this paper is to study the possibility of applying the methods of chromato-mass-spectrometry combined with IR-spectrophotometry in the forensic chemical analysis of trace amounts of unknown poisonous and superpotent substances.

2. MATERIAL AND METHODS

As known, the chromatographic analysis is the most widespread method for the study of narcotic drugs, psychotropic substances and precursors. But recently, due to the high informativeness, sensitivity and selectivity in the world practice of forensic chemical and medical expertise, more preference is given to the methods of chromato-mass-spectrometry and IR spectrophotometry.

The method of gas-liquid chromatography with the mass spectrometric detector is the most optimal which allows to obtain maximum data on the substance (retention time, molecular and fragment ions, peak intensity) present in the trace amounts. Furthermore, the speed, relative simplicity of sample preparation, accuracy and reproducibility of the results raise no doubts.

IR spectrophotometry is applied in various fields of science, including forensic practice. This method of analysis allows to identify organic substances in various aggregative states (gas, liquid, solid). IR spectrophotometry determines the inertia of the substance in a molecule per unit time. Knowing the mass of atoms in the substance allows us to determine the distance between atoms and the angles of their interaction.

IR spectroscopes are used to determine the IR spectrum of the unknown substance. Based on the results of the obtained spectrum, the types of vibrations and bonds of the functional groups of the substance molecule, as well as the structure of the substance, are determined [6, 7].

So, the judicial and investigation authorities for the forensic study provided material evidence, taken from the place of discovery of Mr. A. Dmitriev's corpse. Together with other material evidences, there were also an empty glass and a used empty disposable syringe. The experts were asked the following questions: are there traces of any substances in the glass and syringe; if any, are these substances related to poisonous and superpotent substances? Determine the name of the substances. Are these substances included in the list of narcotic or psychotropic substances?

3. RESULTS AND DISCUSSION

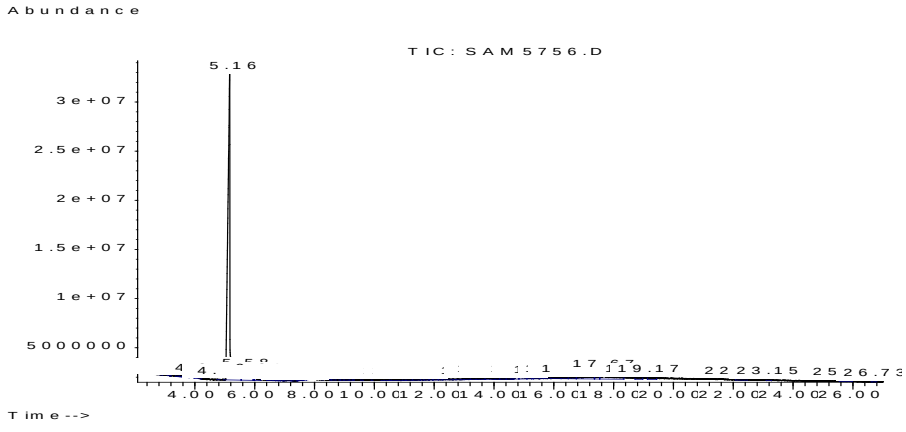
The initial stage in the study of material evidence received for forensic examination - the glass and syringe, in order to detect poisonous, superpotent, narcotic drugs and psychotropic substances, is the extraction of supposed above substances using an organic solvent. For this purpose, the most suitable solvent is ethyl alcohol.

The second stage of the study of trace amounts of unknown substances is the detection of active components and related substances using instrumental methods.

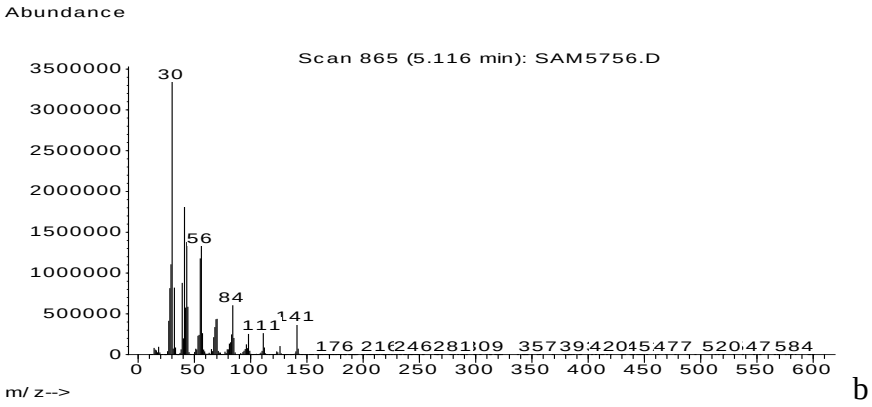
The preparation of samples for analysis: the inner surface of the glass and syringe was washed with ethyl alcohol and then the resulting solution was combined. The obtained wash was evaporated to a volume of 100 μl and used for further analysis.

Chromato-mass-spectrometric study was conducted on AT 5973 gas-chromatography-mass spectrometer (capillary column, 30 mm long, 0.25 mm in diameter, with 5% phenylmethylsiloxane, mass-selective detector) under the following analysis conditions: ionizing electrons energy - 70 eV, injector temperature - 280 °C, furnace temperature from - 150 ° to 280 °C in a programmed mode with a rate of temperature elevation 15 °C per minute, sample volume - 1 μl, vapor pressure of the test substance - 10 mm Hg. 94, analysis time - 20 min, carried gas - hydrogen, flow rate - 2.1 ml/min, in a split mode 10: 1.

Peak identification was carried out by retention time and mass spectra using the device's basic databases. The analysis of the obtained chromatogram and mass spectrum indicated that the mass spectrum of the investigated extract was characterized by the presence of stable fragments, characteristic ions formed along the common pathways of molecular ion fragmentation. Thus, the chromatogram of the extract taken from the glass revealed the main peak with a retention time of 5.16 min and fragmentation ions with m/z 141, 111, 84, 56, 30, corresponding to pregabalin.



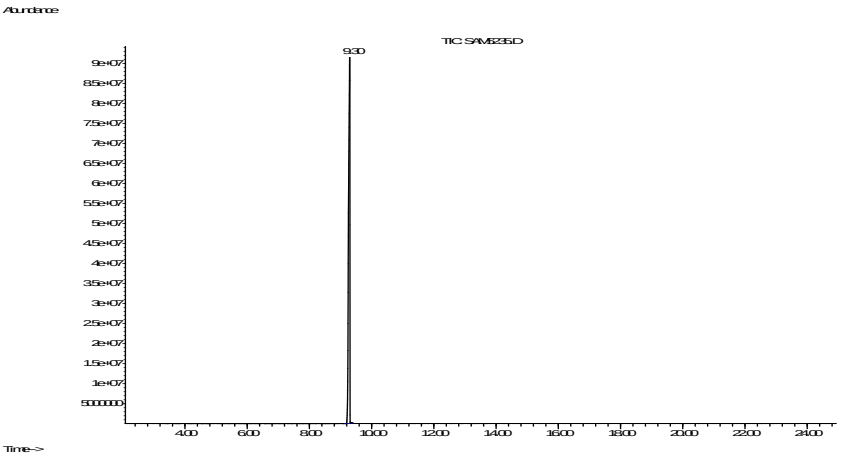
a



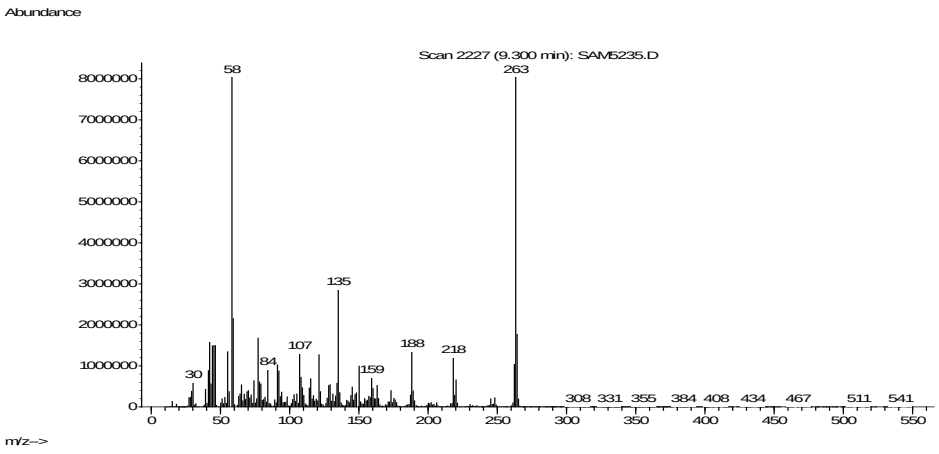
b

Fig. 1. Chromatogram (a); mass spectra of the studied extract from the glass (b)

The chromatogram of the syringe extract showed a major peak with retention time of 9.30 min, and the mass spectrum was characterized by the presence of fragment ions with m/z 218, 188, 159, 135, 107, 84, 58, 30, which corresponding to tramadol.



a



b

Fig. 2. Chromatogram (a); mass spectrum of the studied extract from syringe (b)

The residual washes from the glass and syringe were evaporated to dryness and IR spectral analysis was performed on Agilent Technology FTIR-640 IR spectrometer using an ATR (attenuation total reflectance) attachment under the following analysis conditions: recording range - 2954-422 cm^{-1}, number of scans - 12. The identification of the IR-spectra was carried out on the basis of a comparison of the obtained IR spectra with the standard spectra from the database of the IR spectra library of the device.

Herewith, on the IR spectrum of the traces from the glass we obtained IR spectrum with characteristic passbands in the region of 2952, 1642, 1551, 1389, 1279, 1025, 867, 702, 467 cm^{-1}, due to stretching and deformation vibrations of free and bound hydroxyl (-OH), methyl ($-CH_3$), amine ($-NH_2$-) groups. The functional groups identified by the characteristic passbands are related to the pregabalin structure, which also coincided with the data available in the device's library database.

The analysis result of the obtained IR spectrum indicated that the IR spectrum of the investigated extract was characterized by the presence of characteristic passbands of functional groups of pregabalin structure. The results were also confirmed by comparing them with the data of the device's library database (Fig. 3.).

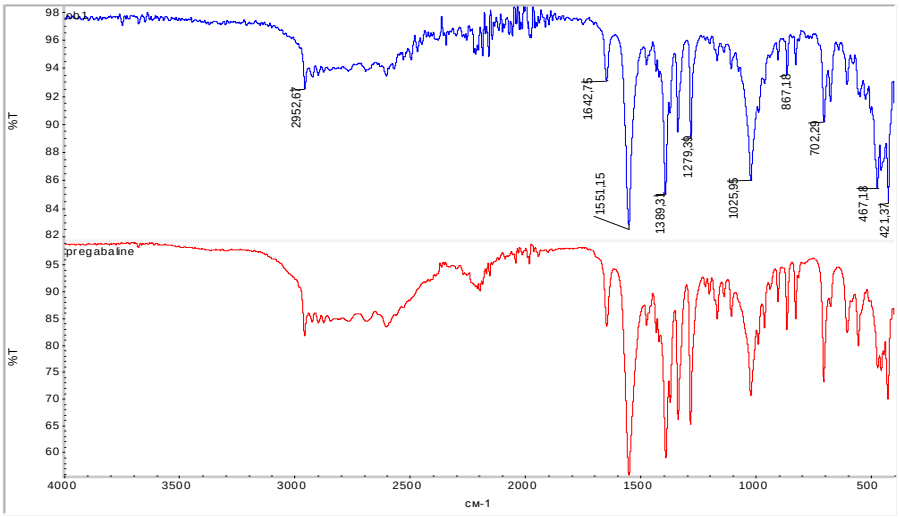


Fig. 3. IR spectra of the dry residue of the investigated extract from the glass and from the device library

Table 1
Indications of the device and its library

Tue Apr 14 15:11:32 2020 (GMT + 05: 00) SEARCH: Spectrum: reg1 Region: 3301.66-748.26 Search type: Correlation List:			
Index	**Match**	**Compound**	**Device Library**
20	80,07	Pregabalin	USER LIB
16563	60,97	1-NITROETHANE-1,1-D2, 99 ATOM % D	Aldrich FT-IR Collection Edition II
316	59,44	Cymel 1123	Coatings Technology

275	57,95	Cymel 114	Coatings Technology
16562	55,57	NITROMETHANE-D3, 99 ATOM % D	Aldrich FT-IR Collection Edition II
2477	55,11	1-NITROPENTANE, 97%	Aldrich FT-IR Collection Edition II
2508	55,00	Enamel,Acrylic	Georgia State Forensic Automobile Paints
2469	54,96	Sodium fusidate; Fusidic acid, Na salt	Toronto Forensic
317	54,84	Cymel 1125	Coatings Technology
2533	54,73	Enamel,Acrylic	Georgia State Forensic Automobile Paints

The IR spectrum of the glass extract contained characteristic passbands of functional groups related to the pregabalin structure.

The IR spectrum of the traces from the syringe showed the IR spectrum with characteristic passbands in the regions of 3303, 2928, 1682, 1581, 1447, 1294, 1239, 1138, 1047, 1010, 778, 723 cm^{-1}, due to stretching and deformation vibrations of free and bound hydroxyl (-OH), methyl ($-CH_3$), methylene ($-CH_2$ -) groups. The functional groups identified by the characteristic passbands were related to tramadol and cellulose structure, which also coincided with the data in the library database of the device.

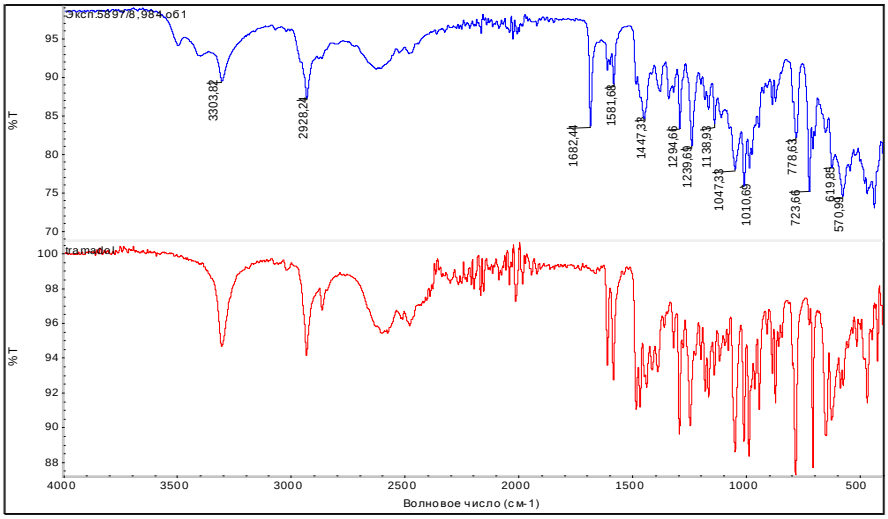


Fig. 4. IR spectra of the studied extract dry residue from the syringe and from instrument library

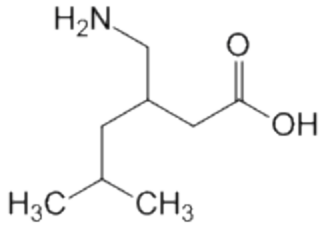
Table 2
Indications of the device and its library

Tue Jun 18 12:26:15 2019 (GMT + 05: 00) SEARCH: Spectrum: Exp. 5897/8, 984 reg1 Region: 3301.66-748.26 Search type: Correlation List			
Index	**Match**	**Compound**	**evice Library**
19	65,68	Tramadol	SER LIB

1760	57,58	Hexyl 4-hydroxybenzoate; Hexylparaben	Toronto Forensic
305	53,01	Propyl 4-hydroxybenzoate	Food Additives
228	51,69	Methyl 4-hydroxybenzoate; Methyl paraben	Food Additives
447	49,93	Diethylpropion .HCl; 2-(Diethylamino) propiophenone .HCl	Toronto Forensic
1757	49,63	Ethyl 4-hydroxybenzoate; Ethylparaben	Toronto Forensic
83	49,25	DIETHYLPROPION HCL INKBR	Georgia State Crime Lab Sample Library
83	49,23	Diethylpropion .HCl	Georgia State Forensic Drugs
495	48,61	3-Methyl-2-buten-1-ol; Prenol	Food Additives
13869	47,29	9-ETHYL-3-CARBAZOLECAR BOXALDEHYDE, 98%	Aldrich FT-IR Collection Edition II

Thus, the results of gas chromatographic analysis with the mass spectrometric detector, IR spectrophotometric analysis of the extract from the glass identified the presence of pregabalin presence.

Structural formula



Name: Pregabalin.

Latin name of the substance:

Pregabalinum (genus Pregabalini)

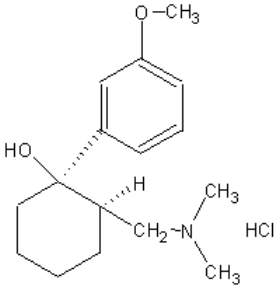
Chemical name:

(S) -3- (Aminomethyl) -5-methylhexenoic acid

Pregabalin which was determined in the glass taken from the crime scene, is the group of antiepileptic drugs, used in cases of neuropathic pain and anxiety disorders. Besides, this compound is available in capsules "Lyrica", "Regapen" as depression reliever. When using it for other purposes or exceeding the dose, acute poisoning may occur, ending by fatality in some cases.

The analysis of the syringe extract using gas chromatography with the mass spectrometric detector, and IR spectrophotometry showed the presence of tramadol.

Structural formula



Name: Tramadol

Latin name of the substance: Tramadolum

Chemical name:
trans - (+ -) - 2- (dimethylaminomethyl) -1- (3-methoxyphenyl) cyclohexanol hydrochloride

Tramadol is an opioid analgesic, used in medical practice for severe pain. In case of it prolonged use in large doses, an acute poisoning can occur, leading to mortality in some cases.

Thus, the complex application of the methods of gas-liquid chromatography with the mass-spectrometric detector and IR-spectrophotometry revealed the presence of pregabalin traces in the examined glass, which is an active component of such preparations as "Gabana", "Algerica", "Neogabin", "Linbag";

Pregabalin, according to the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan No. 818 "On regulation of superpotent substances in the Republic of Uzbekistan" dated September 27, 2019, is included in the list of superpotent substances (55^{th} position) subject to the control on the territory of the Republic of Uzbekistan.

Besides, the conducted analysis revealed tramadol traces in the empty syringe. Tramadol is the active ingredient of the tablets Dromadol, Tramake, Tramal, Ultram, Zamadol, Zydol, and also is included in the list of drugs subject to control on the territory of the Republic of Uzbekistan.

4. CONCLUSION

Thus, in the result of the studies carried out by using gas-liquid chromatography with the mass spectrometric detector and IR spectrophotometry, the complex method was developed for the forensic chemical examination of pregabalin and tramadol trace amounts on the object-carriers.

During the research we established retention time, molecular and fragmentation ions, their intensity, individual fragmentation of pregabalin and tramadol. IR spectra were obtained with characteristic passbands of functional groups related to pregabalin and tramadol structures. It is recommended to use these parameters for the analysis of trace amounts of unknown substances, as well as for establishing the generic and group assignment and the source of origin.

It has been proven that the complex application of these methods, which have high sensitivity, speed and simplicity in use, affords to quickly and accurately identify unknown substances in the composition of micro-objects.

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