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# DEVELOPMENT OF METHODS FOR THE STUDY OF DICYCLOMINE HYDROCHLORIDE IN COMBINATION WITH PARACETAMOL AS AN OBJECT OF FORENSIC-PHARMACEUTICAL EXAMINATION

**Olena Bevz, Igor Sych, Andrii Fedosov, Olha Vislous, Iryna Sych, Olga Kryvanych, Nataliia Kobzar, Lina Perekhoda**

***The aim.*** *Selection and development of methods for the tasks of forensic pharmaceutical examination as case materials suspected of falsification or non-medical use of dicyclomine hydrochloride in combination with paracetamol in the form of tablets.*

***Materials and methods.*** *The study presents the developed methods of detection and identification of dicyclomine by TLC, IR spectroscopy and GC-MS, which were performed using reagents that meet the EP, USP and USPU requirements, Class A glassware and qualified devices.*

*Identification by IR spectroscopy was performed in the range from 500 to 4000 $cm^{-1}$ on the device "Nicolet 380 FT-IR Spectrometer by Thermo Fisher Scientific".*

*TLC was performed on Sorbfil plates for TLC-PET-H-UV and Sorbfil plates for TLC-AF-UV (CJSC "Sorbpolymer", Russia). The following systems were used as mobile phases: dioxane-chloroform-acetone-25 % ammonia solution (47.5:45:5:2.5); toluene-acetone-ethanol-25 % ammonia solution (45:45:7.5:2.5); ethyl acetate-methanol-25 % ammonia solution (17:2:1)). The resulting chromatographic zones were detected by irradiation with UV light and further treatment with color reagents (30 % iron (III) chloride solution; Dragendorff's reagent modified by Munier; Marquis reagent; Froehde reagent; Mandelin reagent; FPN reagent).*

*Analysis by gas chromatography with mass detection was performed using a gas chromatograph with a mass spectrometric detector GCMS-QP2020. Data were analyzed using the program: GCMSsolution, LabSolutions Insight (Shimadzu Corporation, Tokyo, Japan).*

***Results****. For the first time, the conditions for the extraction of dicyclomine hydrochloride from aqueous solutions were studied and the optimal conditions for their isolation as an object of forensic pharmaceutical examination is defined. The method of detection of dicyclomine hydrochloride and paracetamol in the drug "Trigan-D" by the methods of thin-layer chromatography, gas-liquid chromatography and chromato-mass spectrometry was developed, and the detection limits of the substances under study were determined.*

***Conclusion****. The developed methods for dicyclomine hydrochloride in the form of tablets with paracetamol meet the requirements of the current legislation of Ukraine and the Ministry of Justice of Ukraine. The data obtained prove the high sensitivity and reproducibility of the methods and prove the possibility of their introduction into the practice of forensic pharmaceutical examination*

***Keywords****: non-medical use, psychoactive substances, forensic pharmaceutical examination, detection of medicinal substances*

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## 1. Introduction

Recently, several countries have recorded a surge in recreational offer and use of pharmaceutical preparations containing synthetic cholinolytics for unregulated purposes, their abuse and "non-medical" use are manifested by a variety of mental and somatic disorders, behavioural disorders, social degradation [1, 2].

One of these drugs is dicyclomine (Fig. 1), which has been a well-known drug of the grey market for quite a long time. However, it has recently been on the wave of interest in pharmacy substances that there has been a surge in its recreational popularity in the CIS countries, Eastern Europe, and the USA. Dicyclomine is used as an independent object of consumption, and as an adulterating agent of other more expensive psychoactive products [3].

Dicyclomine as a medicinal substance has cholinolytic, myotropic and antispasmodic effects, eliminates spasms of the smooth muscles of the gastrointestinal tract and reduces the pain syndrome caused by it. In therapeutic doses, it has a weak effect on the central nervous system, while in toxic doses it causes the development of bright visual (less often auditory) hallucinations [4].

Dicyclomine is also included in the combined drug "Trigan D" containing 500 mg of paracetamol and

20 mg of dicyclomine hydrochloride. If 1–2 tablets are prescribed for therapeutic purposes depending on the severity of pain, 1–4 times a day, then drug addicts take this drug in an amount of more than 5 tablets at a time. In this case, there may be a pronounced psychomotor agitation, delusional disorders and euphoria accompanied by visual hallucinations [5].

A single dose of about 10 tablets is dangerous for the consumer's mental health. Prolonged use of "Trigan D" in high doses contributes to the development of psychotic disorders and can lead to the development of chronic mental disorders in consumers, accompanied by emptiness, boredom, depression, which quite often lead to suicide [6, 7].

Every year, Ukrainian expert forensic centers receive about 5–8 cases of the drug "Trigan D" as materials seized from persons on suspicion of narcotic substances, as well as 2–3 cases of suspicion of falsification/illegal importation of this drug into Ukraine.

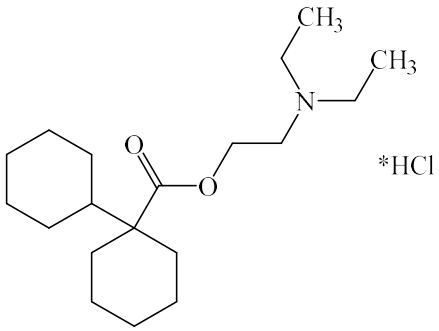



Fig. 1. Structural formula of Dicyclomine hydrochloride

**The aim of the work** was to develop and use the method proposed in cases of a drug reaction of the body to a drug containing dicyclomine as an active pharmaceutical ingredient, withdrawal of the drug during forensic examination, as well as during the pharmaceutical analysis as an identification test. This study was focused on the methods of detection and identification of dicyclomine in forensic-pharmaceutical examination materials using TLC, IR spectroscopy and GC-MS methods.

## 2. Planning (methodology) of research

Taking into account the cases of non-medical use of dicyclomine and the absence of a protocol registered by the Ministry of Justice of Ukraine for conducting a forensic examination of materials with dicyclomine, which showed the prospects of using TLC, IR spectroscopy and GC-MS, which are pharmacopeial methods approved for use in pharmaceutical and forensic analysis, are promising for registration in law enforcement agencies and can be used further in pharmacy and forensic pharmaceutical examination to detect dicyclomine in case materials [8–10].

The research of the article was carried out according to the following Fig. 2.

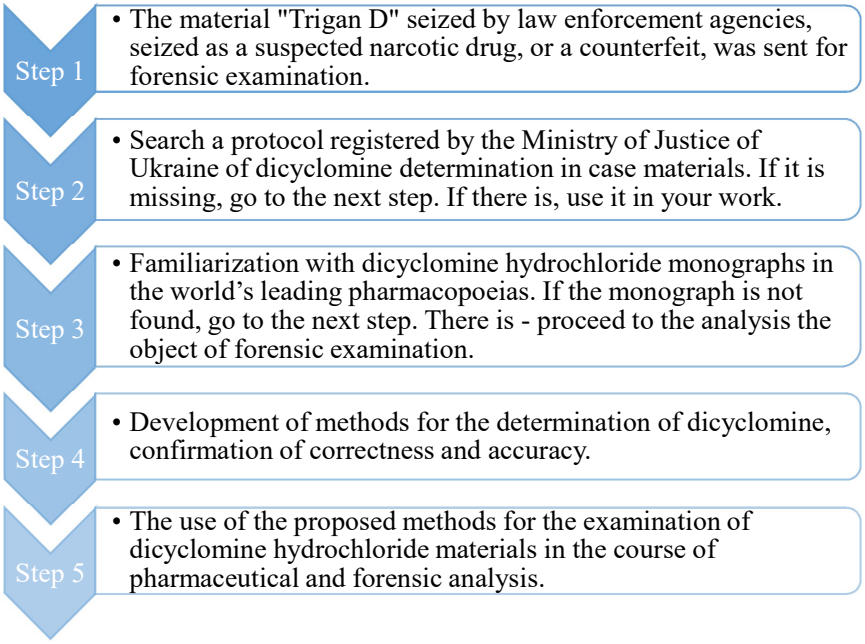



Fig. 2. Scheme of research of dicyclomine hydrochloride materials in pharmaceutical and forensic analysis

## 3. Materials and methods

The study was conducted in period March 2021–January 2022. The studies were carried out on the drug "Trigan D" (c. ET173E9003, "Cadila Pharmaceuticals Limited", India) using the dicyclomine hydrochloride substance, "Haihang Industry Co., Ltd.", China, (the content of the active substance – 99.0 %), and the paracetamol substance, "Zhejiang Kangle Pharmaceutical Co., Ltd.", China, (the content of theactive substance – 99.0 %) as reference samples.

Identification by IR spectrophotometry was performed on a Nicolet 380 IR Fourier spectrometer (Thermo Scientific, USA): the spectrum registration range: – 4000 – 500 $cm^{-1}$, resolution – 4 $cm^{-1}$, the recording speed – 0.6329; amplification–1.0; the number of scans – 4; the scanning rate– 1 mm/s; the scanning mode – in reflected light; a detector – TGS.

To perform the analysis by the TLC method, Sorbfil plates for TLC-PET-H-UV and Sorbfil plates for TLC-AF-UV (CJSC "Sorbpolymer", Russia) with the sizes of 10x15 cm were used. Samples of the extracts studied were applied to the plate using a 10 µl micro-syringe (Agilent Technologies, USA). The resulting chromatographic zones were detected by irradiation with UV light (UVS 254/365 chromatographic irradiator (CJSC "Sorbpolymer", Russia) and further treatment with color reagents (1 – 30 % iron (III) chloride solution, 2 – Dragendorff's reagent modified by Munier, 3 – Marquis reagent, 4 – Froehde reagent, 5 – Mandelin reagent, 6 – FPN reagent).

Mobile phases (dioxane-chloroform-acetone-25 % ammonia solution (47.5:45:5:2.5); toluene-acetone-ethanol-25 % ammonia solution (45:45:7.5:2.5); ethyl acetate-methanol-25 % ammonia solution (17:2:1)) were pre-

pared immediately before use by mixing the components in the required proportions. The walls of the chromatographic chamber were laid with filter paper. The saturation time of the chromatographic chamber was 25 minutes.

The GC analysis was performed using a HP-5 chromatographic column (Agilent Technologies, USA) with the length of 30 m, the inner diameter of0.25 mm, the film thickness of the stationary liquid phase of 0.25 µm.

The non-polar stationary liquid phase was (5 %-phenyl)-methylpolysiloxane.

Nitrogen was used as a carrier gas, while hydrogen and air were used as auxiliary gases.

The sample was injected into the chromatographic column automatically by an AOC-20i autoinjector using a 10 µl Shimadzu AOC micro-syringe.

Chromatographic information was processed using the "Labsolution" software.

The GC-MS analysis was performed on a Shimadzu GCMS-QP 2020 gas chromatography-mass spectrometer. The detector was a quadrupole mass spectrometer operating in the mode of ionization by an electron shock of 70 eV.

A HP-5MS capillary quartz column (Agilent Technologies, USA) with the length of 30 m and the internal diameter of 0.25 mm was used in the analysis. Helium was used as a carrier gas. The sample was injected manually with a 10 µl Shimadzu micro-syringe. The peaks were identified using the NIST 17.0 database, Wiley (11th Edition), SWGDRUG 3.7.

The mass spectra were considered to be identified when the mass spectra of the substance studied coincided with the library one with a similarity coefficient exceeding 90 %.

All reagents and glassware used for the study complied with the requirements of ISO and the world's leading pharmacopoeias.

**4. Result**

The presence and structure of dicyclomine in the study object was determined by the characteristic absorption bands using IR spectroscopy (Fig. 3), by the presence of a spot at the level of the spot of the reference sample using TLC and based on the coincidence of the retention time and the mass fragmentation of the peaks detected on chromatograms using GC-MS (Fig. 4–9).

The IR spectrum of dicyclomine hydrochloride isolated from the drug "Trigan D" (Fig. 3) had the main absorption bands of 1729, 1211, 1105, 1126, 1172 $cm^{-1}$; it completely coincided with the IR spectrum of the reference sample of dicyclomine hydrochloride from electron base of IR-spectra.

To identify the substances under study by TLC, their behaviour in chromatographic systems widely used in the chemical and pharmaceutical analysis of the medicinal poisons group was studied. Since paracetamol was included in the composition of the drug "Trigan D" in addition to dicyclomine hydrochloride, therefore, a standard paracetamol solution was also studied in the systems mentioned. On the start line of chromatographic plates, 10 µl of the study objects ("Trigan D" tablets) and standard solutions of dicyclomine hydrochloride and paracetamol in chloroform with the concentration of 1 mg/ml were applied. The plates were dried and placed in chambers pre-saturated with the mobile phase and chromatographed in the following solvent systems (Table 1) [11].

The results obtained indicated that the substances studied had satisfactory Rf values in all selected systems on both types of Sorbfil plates.

When detecting the adsorption zones of dicyclomine hydrochloride and paracetamol on chromatographic plates the ratio of the substances under research to several potential detecting agents was studied (Table 2).

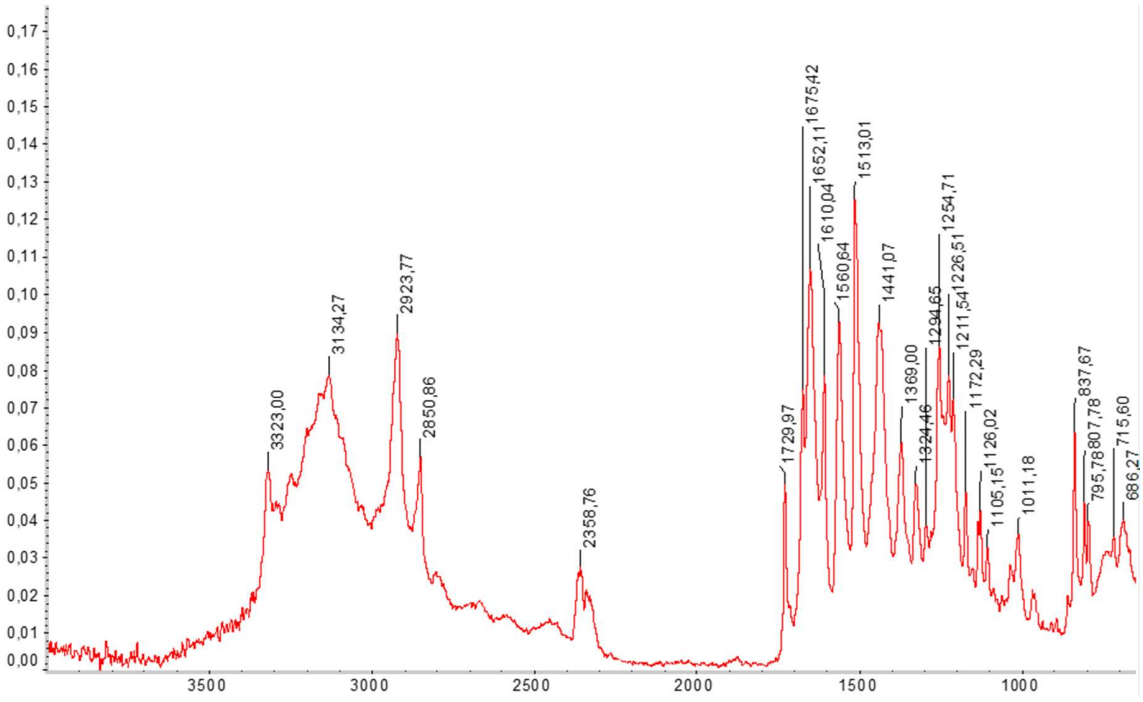



Fig. 3. IR-spectra of "Trigan D"tablets

Table 1

The Rf values of the substances studied

| System | Dicyclomine hydrochloride | Paracetamol |
|---|---|---|
| Sorbfil plates for TLC-PET-H-UV | | |
| 1 | 0.79 | 0.49 |
| 2 | 0.84 | 0.53 |
| 3 | 0.80 | 0.66 |
| Sorbfil plates for TLC-AF-UV | | |
| 1 | 0.77 | 0.48 |
| 2 | 0.86 | 0.49 |
| 3 | 0.79 | 0.62 |

Table 2

The results of detection of the substances studied

| Compound | Revealing reagent | Result |
|---|---|---|
| Dicyclomine hydrochloride | UV light (254, 365 nm) | – |
| | Dragendorff's reagent modified by Munier | A spot of orange-brown color |
| | Mandelin reagent | – |
| | Marquis reagent | – |
| | Froehde reagent | – |
| | Iron (III) chloride solution, 30 % | – |
| | FPN reagent | – |
| Paracetamol | UV light (254, 365 nm) | The absorption region is dark blue at 254 nm |
| | Dragendorff's reagent modified by Munier | A spot of orange-brown color |
| | Mandelin reagent | – |
| | Marquis reagent | – |
| | Froehde reagent | – |
| | Iron (III) chloride solution, 30 % | A spot of blue color |
| | FPN reagent | – |

When the plate was irradiated with UV light (the wavelength of 254 nm), the absorption zone was observed only for paracetamol. Dicyclomine hydrochloride was not detected in UV light. It was also found that dicyclomine hydrochloride did not give a characteristic staining when interacting with Marquis, Froehde, Mandelin, and FPN reagents, as well as with 30 % iron (III) chloride solution. It was found that when treating plates with Dragendorff's reagent paracetamol and dicyclomine were detected in the form of orange-brown spots. Although Dragendorff's reagent was not specific to the substances under study, it gave a clear analytical effect and had a high sensitivity to the substances being analyzed.

The optimal way to detect the components of the drug "Trigan D" consisted in step-by-step processing of the chromatogram using:

– irradiation in UV light (254 nm) – a dark blue absorption zone (paracetamol) was observed;

– 30 % iron (III) chloride solution –paracetamol was detected in the form of a blue spot;

– Dragendorff's reagent– dicyclomine was detected in the form of an orange-brown spot, the color of the paracetamol spot changed from blue to orange-brown.

When processing the chromatogram by the TLC method with the Dragendorff's reagent, dicyclomine hydrochloride was detected at a content of 1 μg and more in the spot (detection limit – 1 μg).

The results of GC-MS were as follows. The study was performed on a gas chromatograph with a Shimadzu GCMS-QP2020 mass-selective detector on an HP-5 capillary non-polar column. The sample (1 μl) was injected manually with a split ratio of 50:1. The mass detector operated in the mode of scanning electron impact spectra at 70 eV in the range from 50 to 550 amu.

When developing the method the flow rate of the carrier gas, the temperature conditions of the injector, the chromatographic column and various extraction methods (chloroform, ethyl alcohol and water) were varied. The analysis was performed using the flow rate of a carrier gas from 0.7 to 2.0 ml/min. The optimal flow rate was 1 ml/min. At a lower rate of the carrier gas the broad chromatographic zones of the substances studied were obtained, and at a flow rate of more than 1 ml/min the inefficient separation of analytes with biomatrix ballast substances was observed. In the process of determining the optimal temperature of the injector, the following values were tested: 200 °C, 210 °C, 220 °C, 230 °C, 240 °C, 250 °C, 260 °C, 270 °C. When using the temperature range of 200–240 °C the broad peaks of unsatisfactory shape were observed. At temperatures above 250 °C there was a partial decomposition of substances in the evaporator, and it led to the appearance of additional interfering peaks on the chromatogram. The column temperature conditions were selected in such a way that the peak shape and separation with co-extractive substances were satisfactory for dicyclomine hydrochloride.

Chromatograms of ethanol and chloroform solutions contained two peaks corresponding to the main components of the drug "Trigan D". In both cases, chromatograms had signals of by-products that could not be identified. The use of hexane as an extractant gave a negative result — the chromatogram did not contain signals of active substances.

Characteristic ions in the mass spectrum of dicyclomine hydrochloride are: 86, 71, 99, 58, 55, 56, 100, 87 m/z (data are given in decreasing order of m/z).

Characteristic ions in the mass spectrum of paracetamol are: 109, 151, 43, 80, 108, 81, 53, 52 m/z (data are given in decreasing order of m/z).

It should be noted that the ratio of the peak areas of substances when using ethanol and chloroform differs markedly from the quantitative ratio of these substances in the drug "Trigan D" in favour of paracetamol. This fact indicates that the above methods of sample preparation are not suitable for the quantitative analysis, and in the case of hexane – for the analysis in principle, since they do not provide complete and uniform extraction of active substances from the tablet mass. The use of a mass-selective detector makes it possible to correlate chromatographic signals with specific substances based on the mass spectrum data.

The limit of detection of dicyclomine hydrochloride GCMS according to the proposed method was 0.05 μg/ml.

The quantitative determination of active substances as objects of forensic examination does not make any sense since dicyclomine hydrochloride and paracetamol are absent in "The list of narcotic drugs, psychotropic substances and precursors" approved by the Resolution of the Cabinet of Ministers No. 770 of 06.05.2000.

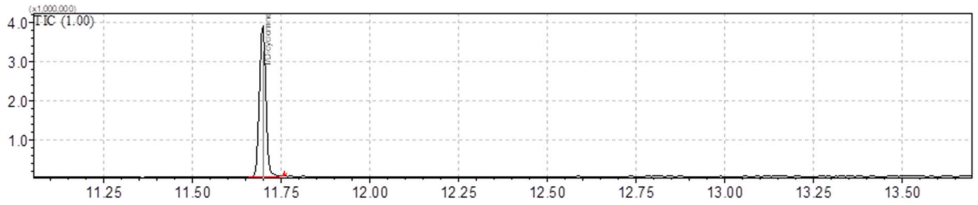



Fig. 4. The chromatogram of dicyclomine hydrochloride extraction from chloroform

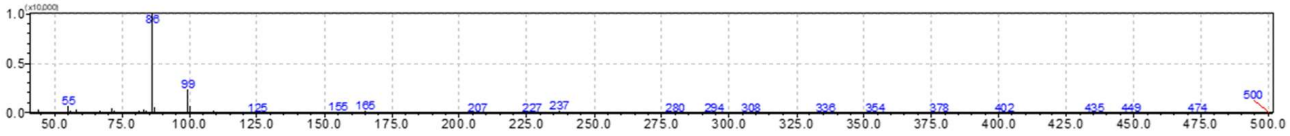


Fig. 5. The mass spectrum of dicyclomine hydrochloride

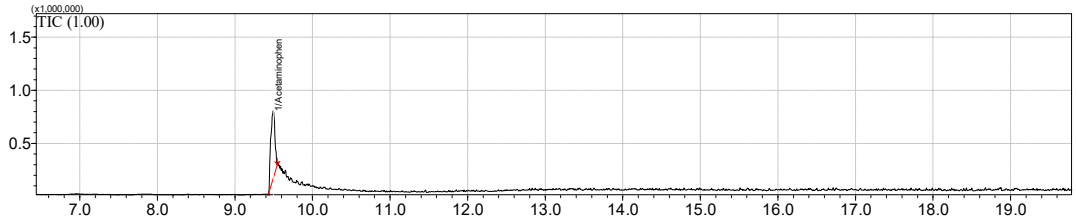



Fig. 6. The chromatogram of paracetamol isolated from a "Trigan D" tablet

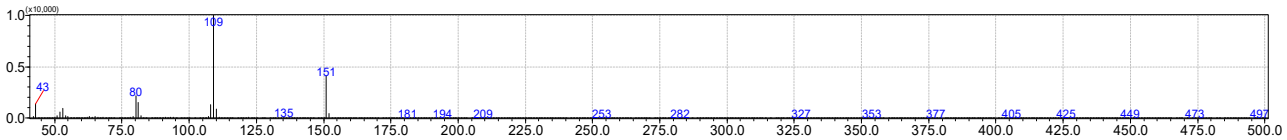


Fig. 7. The mass spectrum of paracetamol

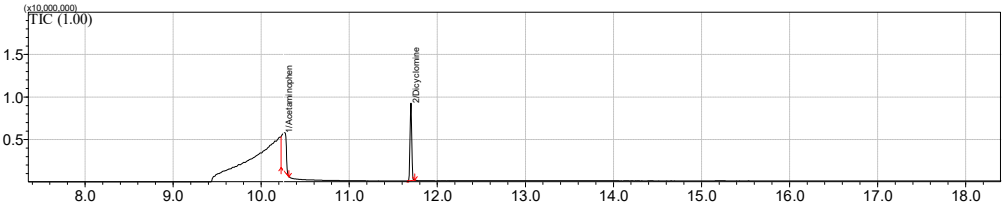
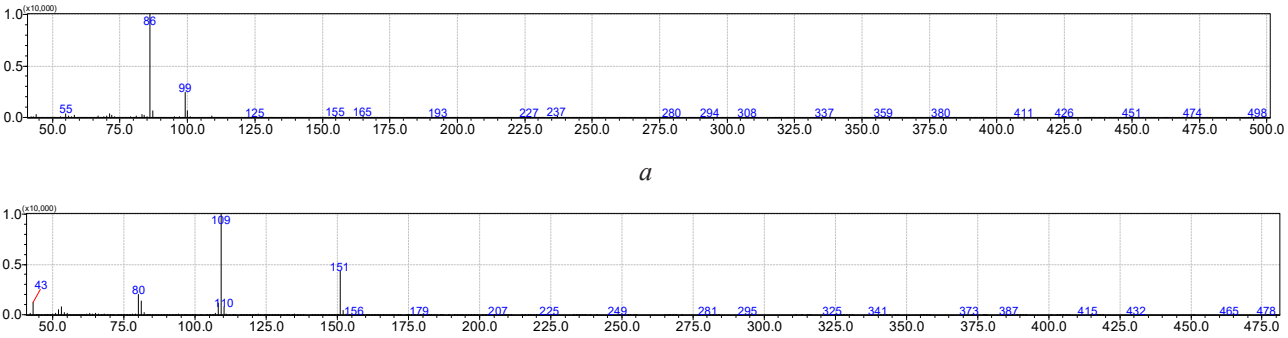



Fig. 8. The chromatogram of the drug "Trigan D"



*a*

*b*

Fig. 9. The mass spectra of: *a* – dicyclomine hydrochloride; *b* – paracetamol

**5. Discussion**

Literature review reveals that methods have been reported for analysis of dicyclomine hydrochloride in bulk and pharmaceutical formulation by UV spectrophotometry [12], colorimetry [13] and voltammetry [14] either alone or in combination with other drugs. Quantification of dicyclomine hydrochloride in blend with other drugs using UV spectroscopy [15], TLC [16] and LC [17, 18] was also reported.

If we consider the methods of colorimetry and voltammetry, then they are unsuitable for solving the tasks of forensic examination, since they are intended for the quantitative determination of dicyclomine hydrochloride, which is not mandatory for forensic research.

UV spectrophotometry is not a sufficiently selective method, it requires the use of a standard sample, and it is not always possible to detect an object of study in a mixture with other pharmaceutical components, these are disadvantages in conducting an examination of materials in a forensic analysis, therefore, we did not consider it as an alternative method for these purposes.

To date, TLC and IR spectroscopy methods are rapidly becoming routine analytical methods due to several advantages associated with low operating costs, high sample productivity and the need for minimal sample preparation.

Unlike LC, the main advantage of TLC is that several samples can be treated simultaneously using a small amount of mobile phase; thus, the analysis time and cost are reduced.

There are literature data on the determination of dicyclomine hydrochloride by GC-MS and TLC methods from a mixture with other pharmaceutical ingredients (like tropicamide, in biological fluids, for the purpose of forensic toxicological analysis.

For these purposes, selected methods that are listed of the methods International Association of Forensic Toxicologists, which are approved and meet international requirements both for toxicological analysis and can be used for forensic pharmaceutical examination [19].

However, the above-mentioned methods have not yet been verified on the basis of forensic pharmaceutical examination materials, which are being considered on suspicion of falsification or non-medical use of dicyclomine in combination with paracetamol in the form of "Trigan D" tablets, considering the presence of auxiliary substances.

During the forensic pharmaceutical analysis TLC method using various eluents was proposed, which was approved by the International Association of Forensic Toxicologists and Detection in UV Light (254 nm) and Dragendorf reagent. The interchangeability of eluents and detection pathways has been confirmed. The advantage of this method is the ability to identify the substance and determine impurities without the use of expensive equipment, ease of execution and speed of analysis [16].

To conduct IR spectroscopy, only the object to be analyzed and the reference sample are sufficient. The studies conducted have proven that these methods are suitable for the analysis of dicyclomine in mono- and combined medicinal products for the purpose of identification in the quality control and the forensic pharmaceutical examination [20].

During our research, an alternative method for the identification of dicyclominehydrochloride in the presence of paracetamol – GC with a Shimadzu GCMS-QP2020 mass spectrometric detector has been proposed. The method of carrying out and the gas chromatographic and mass spectral characteristics of substances described can be used for their qualitative identification of the study object. In addition, the proposed GC-MS method has an advantage over the LC method, which is described in the literature, due to the absence of many toxic reagents, and therefore is more environmentally friendly and economical [21].

**Study limitations.** The proposed methods for determination of dicyclomine hydrochloride can be used considering the modern equipment of forensic laboratories.

**The prospects for further research.** The proposed methods for determining dicyclomine hydrochloride will be submitted for consideration for further use in forensic-pharmaceutical examination.

**6. Conclusions**

For the first time, the conditions for the extraction of dicyclomine hydrochloride from aqueous solutions were studied and the optimal conditions for their isolation as an object of forensic examination were determined.

The optimal conditions for the separation of dicyclomine hydrochloride and paracetamol were studied by TLC on Sorbfil plates, the detection method was substantiated, and the sensitivity was determined.

Methods for the detection of dicyclomine hydrochloride and paracetamol in the drug "Trigan-D" by the methods of TLC, GLC and chromato-mass spectrometry were developed, and the detection limits of the substances under study were determined.

Physicochemical techniques for the detection of dicyclomine hydrochloride and its traces in samples in a laboratory have been developed and tested in National Scientific Center "Hon. Prof. M. S. Bokarius Forensic Science Institute". The proposed methods meet the requirements of current legislation of Ukraine. The obtained results demonstrate that the methods could be used for the study of dicyclomine hydrochloride for forensic and pharmaceutical analysis.

**Conflict of interests**

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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