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Identification of The Composition of Substances Isolated from A Biological Fluid (Urine) Using IR Spectrophotometry and Gas Chromatography–Mass Spectrometry Methods

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Abstract: The article discusses biological fluid samples submitted for forensic examination and the specific features of identifying unknown substances in their composition using methods available in forensic chemical laboratories.

Keywords: Examination, tropicamide, biological fluid, sample, method, gas chromatography–mass spectrometry, IR spectrophotometry, spectrum.

Introduction: Relevance of the study: In our country today, cases of the use of narcotic drugs, along with psychotropic and potent substances, among young people are increasingly being observed. In particular, the illegal consumption of psychotropic and potent substances by minors or individuals with health impairments is contributing to the breakdown of young families. A significant number of various criminal offenses are also being committed by young people.

Despite the fact that all necessary conditions have been created in our society for the healthy physical and mental development of young people, as well as for obtaining modern education and professions, their involvement in crimes related to such substances and their descent into drug abuse remains a troubling issue. In order to prevent such problems and combat crime in society, extensive scientific and practical research is being carried out in the field of forensic expertise.

In recent years, one of the substances frequently encountered in forensic practice is tropicamide, which, despite being an ophthalmic drug, is misused for non-medical purposes. The illicit use of this substance, as well as its combined use with other psychoactive substances, leads to serious adverse consequences.

The examination of micro-quantities of physical evidence submitted for expertise (in the range of 0.1–0.5 mg) has its own specific features. These are related to the extremely small amount of the substance, the necessity to improve analytical methods, the variety of forms in which such substances are encountered, the diversity of carrier materials, and the interaction between trace amounts of substances and their

carriers.

Micro-traces of physical evidence are often used in investigative processes and forensic practice as important sources of information regarding the circumstances of criminal cases. This is because the nature of small quantities of substances on carrier objects, the location of the micro-sample, and the type of carrier material play a crucial role in resolving many investigative questions.

Experimental data show that the minimum required amount for analysis is approximately 1 mg for solid substances and 1 ml for liquid samples. The examination of such small quantities requires a high level of attention and precision from specialists. Therefore, strict requirements are imposed on the instruments and methods used for diagnostic and identification purposes.

The small quantity of substances, their physical state, and the complex nature of their distribution on carrier objects make their detection particularly challenging. Nevertheless, determining the nature of micro-trace evidence, their group affiliation, and their origin is of significant importance for investigative authorities in correctly assessing the circumstances of a case.

Aim of the study: The main aim of this study is to evaluate the possibilities of using modern, highly sensitive analytical methods available in the Central Laboratory for determining the composition and nature of carrier materials and trace amounts of unknown substances submitted for forensic examination.

METHODS

In this study, modern physicochemical analytical methods were applied to identify and determine tropicamide in samples obtained from biological fluids. Particular attention was paid to the stages of preliminary sample preparation, extraction, and purification. Subsequently, the obtained samples were comprehensively analyzed using chromatographic and spectroscopic methods to assess the qualitative and structural characteristics of the substance. The applied methods are characterized by high sensitivity and accuracy, ensuring reliable detection of even trace amounts of substances.

For the extraction of tropicamide from urine, a sample (10 mL) of urine from a healthy individual was used. A standard solution of tropicamide containing 1 mg of the substance was added (1 mL), and the mixture was left to stand for 1 hour. After incubation, the sample was treated with 0.1 M hydrochloric acid to adjust the pH to 4.0. Subsequently, 2 mL of 25% ammonium sulfate solution was added, followed by the addition of 10 mL of diethyl ether as an organic solvent. The mixture was vigorously shaken using a mechanical shaker for 10 minutes. Then, it was centrifuged at 3000 rpm for 5 minutes to precipitate protein substances. After centrifugation, the organic layer was carefully separated. The remaining aqueous phase was re-extracted twice with 10 mL portions of diethyl ether. All organic extracts were combined.

The combined diethyl ether extract was dried by passing it through filter paper containing 5 g of anhydrous sodium sulfate. The solvent was then completely evaporated to obtain a dry residue. The obtained residue was dissolved in 5 mL of 95% ethanol and purified using thin-layer chromatography (TLC) to remove interfering substances.

In the second stage of the study, infrared spectrophotometry and gas chromatography–mass spectrometry (GC-MS) methods were applied. Infrared spectrophotometry is characterized by its simplicity, high sensitivity, and rapid sample preparation. The GC-MS method provides high sensitivity and accuracy and is capable of detecting even very small amounts of analytes in complex mixtures.

RESULTS

1.Although chromatographic analysis remains the most widely used method for the determination of narcotic drugs, psychotropic substances, and potent agents, in recent years infrared spectrophotometry has also become an effective technique in forensic chemical practice due to its high information content, sensitivity, and selectivity. Infrared spectrophotometry has become one of the principal methods for studying substances of various chemical nature, including pharmaceutical compounds and narcotic substances. In forensic analysis, this method is used to determine the nature of unknown materials and to confirm similarities or differences between substances with similar chemical structures.

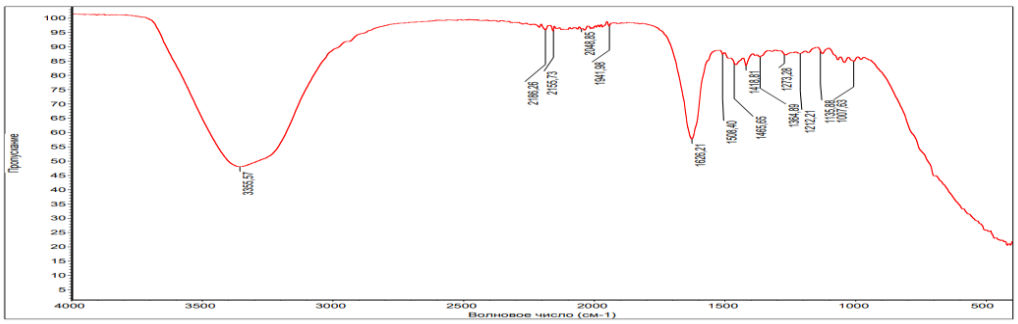
To determine the presence or absence of controlled substances in the submitted sample, the prepared dry residue was analyzed using an Agilent Technology FTIR-640 infrared spectrophotometer. The analysis was carried out under the following conditions: spectral range 4000–400 cm^{-1} and 40 scans.

The obtained infrared spectrum showed characteristic absorption bands at 1632, 1601, 1453, 1414, 1263, 1044, 789, and 701 cm^{-1}. These bands correspond to the stretching vibrations of functional groups such as hydroxyl (–OH), methyl ($–CH_3$), methylene ($–CH_2–$), and carboxyl (–COO–) groups.

The number, intensity, and overall pattern of the absorption bands in the obtained spectrum were found to be in full agreement with the reference spectrum of

tropicamide (Tropicamide) available in the computer spectral library. This confirms the presence of tropicamide in the examined sample.

The obtained infrared spectra are presented below.



1-Figure. Infrared spectroscopic results of tropicamide isolated from urine

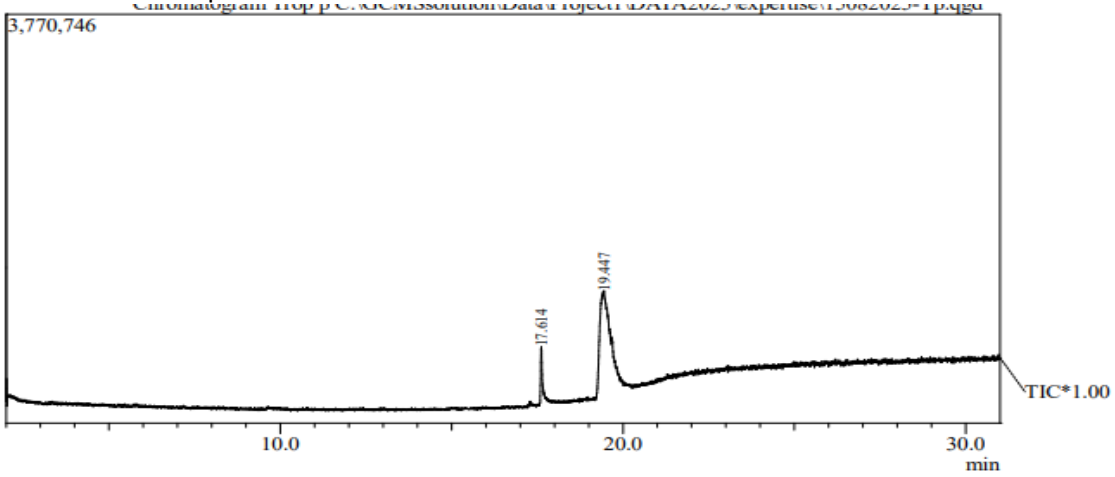
The identification of the obtained IR spectra was carried out by comparison with reference spectra available in the instrument's spectral library. The results of the analysis showed that the infrared spectra of tropicamide isolated from biological fluid (urine) are consistent with the IR spectra of the standard tropicamide sample.

2. Gas chromatography–mass spectrometry (GC–MS) is one of the most widely used physicochemical methods in the analysis of organic compounds due to its high sensitivity, accuracy, and particularly its ability to detect trace amounts of analytes in complex mixtures. In addition, this method is widely applied in cases where the examined substance is unknown or when reference standards are unavailable, as well as for the identification of toxic metabolites formed in the organism as a result of drug metabolism.

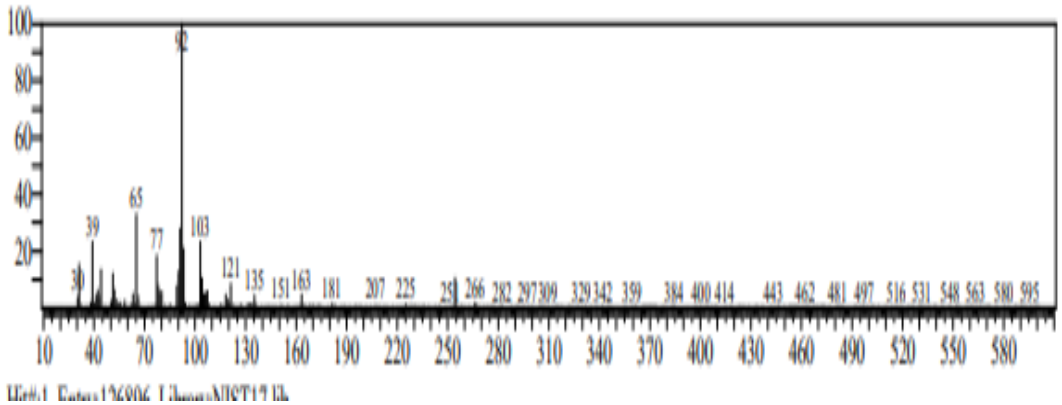
The experiments were performed using a SHIMADZU GCMS-QP2020 gas chromatography–mass spectrometry system. The following analytical conditions were established: a 30 m long metal capillary column with an internal diameter of 0.25 mm, coated with a 0.25 µm film of 5% phenylmethylpolysiloxane in dimethylpolysiloxane; injector temperature 250°C; oven temperature programmed from 150°C to 280°C at a rate of 20°C/min, followed by an additional ramp of 10°C/min up to 280°C; helium was used as the carrier gas with a total flow rate of 118.2 mL/min; injection volume 1 µL; split ratio 1:50; total analysis time 25 minutes.

For the preparation of the standard solution, an accurately weighed amount (5 mg) of tropicamide reference standard was dissolved in 5 mL of 96% ethanol in a 25 mL volumetric flask and diluted to the mark with the same solvent. An aliquot of 1 µL was injected into the chromatographic system.

Under these conditions, the chromatogram showed a characteristic peak for tropicamide at a retention time of 18.57 minutes. The mass spectral analysis of this peak revealed fragment ions with m/z values of 65.92, 103, 121, 163, and 254. The obtained chromatographic and mass spectral data were compared with those in the computer database, and a full match with the structure of tropicamide was confirmed. These results are in full agreement with the principles of gas chromatography–mass spectrometry.



A)



B)

Figure 2. Results of tropicamide extraction from urine (GC–MS chromatogram (A) and mass spectra (B))

Thus, based on the gas chromatography–mass spectrometry investigation of the biological fluid sample submitted for examination, the retention time, molecular and fragment ions, their relative intensity values, and characteristic fragmentation patterns of the molecule were determined.

On the basis of these characteristics, the trace amount of tropicamide present in the sample was identified, and its nature, group affiliation, and classification were established. The obtained results are recommended for use in forensic chemical practice as well as in the resolution of criminal case-related issues.

Tropicamide is a synthetic pharmaceutical compound with anticholinergic activity, widely used in modern ophthalmological practice. Chemically, it belongs to the amide class, and its chemical name is N-ethyl-3-hydroxy-2-phenyl-N-(pyridin-4-ylmethyl)propanamide. Its international nonproprietary name is tropicamide, with the molecular formula $C_{17}H_{20}N_2O_2$ and a molecular weight of 284.35 g/mol.

Regarding its physicochemical properties, tropicamide appears as a white or almost white crystalline powder. It is sparingly soluble in water but readily soluble in ethanol, chloroform, and other organic solvents. The compound is relatively unstable to external factors, particularly light exposure, which requires special attention during storage and analytical procedures. Its melting point is typically in the range of 96–104°C.

Tropicamide is known under various trade names, including Midriacyl, Tropicacyl, Tropicamidum, and Mydriacyl, and is mainly produced in the form of eye drops (0.5% and 1% solutions).

Pharmacologically, it acts as a muscarinic receptor antagonist, inducing pupil dilation (mydriasis) and temporary paralysis of accommodation (cycloplegia). Therefore, it is widely used in ophthalmic diagnostics, including fundus examination, refractive error assessment, and other clinical procedures.

However, the non-medical or uncontrolled use of tropicamide may lead to adverse effects on the central nervous system and may cause intoxication. In some cases, its illicit use in combination with other psychoactive substances has been reported, which further emphasizes the importance of its detection and identification in forensic chemical practice.

CONCLUSIONS

Based on the results of the conducted analysis, tropicamide present in the biological fluid samples was successfully identified using modern, highly sensitive physicochemical methods. In particular, infrared spectrophotometry proved effective in identifying characteristic spectral features of functional groups, while gas chromatography–mass spectrometry ensured reliable identification through retention time, molecular ions, and fragment ions.

The applied methods demonstrated relatively simple sample preparation, high sensitivity, and accuracy, confirming their practical suitability for the extraction and identification of tropicamide from biological matrices.

These results indicate that the applied methods can be widely used in forensic chemical practice for the identification and determination of tropicamide.

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