RESEARCH PAPER

Development of an Analytical Method for Mephedrone and Methamphetamine Using Gas Chromatography-Mass Spectrometry (GC-MS)

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Abstract

This article presents the properties of synthetic psychostimulants mephedrone and methamphetamine as well as the results of developing gas chromatography–mass spectrometry (GC-MS) methods for their identification from biological matrices. In the study, substances extracted from biological samples such as blood, urine, and liver were identified using standard reference materials. For each substance, the characteristic retention time and mass of ion fragments were determined. It was established that the developed method offers high accuracy and sensitivity.

Keywords: mephedrone, methamphetamine, synthetic narcotics, chromatography, toxic effects, forensic medical examination, gas chromatography–mass spectrometry (GC-MS).

How to cite this article: Uktamovna AN, Abidovich YZ, Alisherovna ZD, Ismatovna NM. Development of an Analytical Method for Mephedrone and Methamphetamine Using Gas Chromatography-Mass Spectrometry (GC-MS). Int J Drug Deliv Technol. 2026;16(22s): 588-592. DOI: 10.25258/ijddt.16.22s.70

INTRODUCTION

In recent years, there has been a sharp increase worldwide in the illicit trafficking and abuse of psychoactive substances, particularly synthetic amphetamine-type drugs such as mephedrone and methamphetamine. Due to their potent psychostimulant properties, rapid effects, and relative affordability, these substances are becoming increasingly widespread, especially among young people. This poses significant challenges not only for socioeconomic factors but also for healthcare and law enforcement professionals [1].

According to data published by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2023, over 6 million people in Europe alone had used synthetic stimulants at some point in their lives in 2022.

According to the Ministry of Internal Affairs of the Republic of Uzbekistan, the proportion of crimes related to synthetic narcotics has doubled in the last 3 years [6].

According to the World Health Organization and the United Nations Office on Drugs and Crime (UNODC), more than 70% of deaths related to amphetamine-type substances are due to incorrect dosing, mixed substance use, and lack of timely medical care [1].

The study of mephedrone and methamphetamine from the perspective of toxicological chemistry and forensic toxicology is currently relevant. This is because these substances have a strong neurotoxic effect on the human body, causing severe pathological changes in the cardiovascular, nervous system, and other vital organs [2]. Due to the incompletely understood toxicokinetic and toxicodynamic properties of these substances, there are several problems in accurately diagnosing and treating intoxication cases in medical practice [3].

At the same time, there is a growing need in the Republic of Uzbekistan to develop accurate, reliable, and highly sensitive modern analytical methods for isolating and analyzing these substances from biological fluids (blood, urine) and tissues. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) have been shown to be important in toxicological chemistry and forensic medical examination, and necessary results can be obtained when they are used for diagnostic purposes in forensic and clinical practice [4].

Therefore, improving the methods for isolating and identifying mephedrone and methamphetamine from various objects is a relevant issue today with not only scientific but also practical significance.

MATERIALS AND METHODS

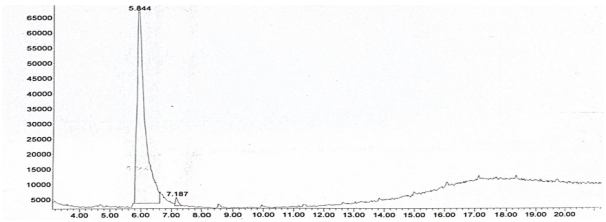
The experiments were conducted on a "XEVO Ta-GC/GC-8890" gas chromatograph-mass spectrometer. As a result of the research, the following conditions were selected: a metal capillary column with a length of 30 m and an inner diameter of 0.25 mm, the inner walls of which are coated with a 5% solution of phenylmethylsiloxane in dimethylsiloxane with a thickness of 0.25 μm; injector temperature 200°C; column thermostat temperature is increased from 200°C to 300°C at a rate of 10°C/min; carrier gas - helium, total gas flow rate 10 ml/min; injected sample volume 1

µl; in a 1:50 flow splitting mode; the analysis time is 25 minutes.

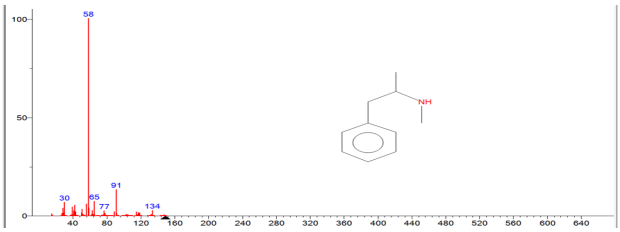
To prepare the standard sample solution, an exact amount (5 mg) of each substance standard sample was weighed and transferred to a 25 ml volumetric flask, dissolved in 5 ml of 96% ethyl alcohol, and the solvent was added to the mark of the flask. A volume of 1 µl was taken from the sample and sent to the chromatography column.

RESULTS AND DISCUSSIONS

Under these conditions, peaks appeared on the chromatogram with retention times of 5.844 and 3.69 minutes. In the mass spectrum, mephedrone fragment ions with a mass of 30, 58, 65, 77, 91, 134 m/z and methamphetamine fragment ions with a mass of 30, 58, 65, 77, 91, 134 m/z, corresponding to the peaks in the chromatogram, were detected and identified as mephedrone and methamphetamine, respectively. The obtained chromatograms and mass spectra of mephedrone and methamphetamine were compared with the indicators in the database of the computer library, and it was found that their structure corresponds to the structure of mephedrone and methamphetamine (Figures 1-2).

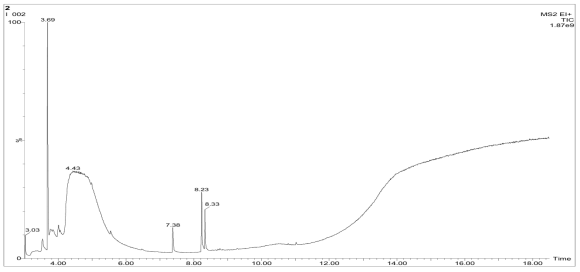


(a)

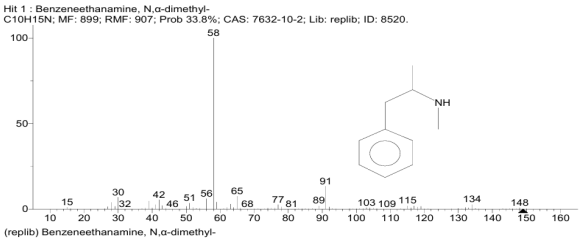


(b)

Figure 1. Chromatogram (a) and mass spectra (b) of the mephedrone standard sample solution.



(a)



(b)

Figure 2. Chromatogram (a) and mass spectra (b) of the methamphetamine standard sample solution.)

For the analysis, initially confiscated physical evidence - two unknown powders - was taken, and 100 mg was precisely weighed from each and transferred to 25 ml volumetric flasks, to which 5 ml of 96% ethyl alcohol and one drop of 25% ammonia solution was added to equilibrate the substance's environment, and a solution was prepared. The prepared solutions were treated in a "SONOREX" ultrasonic bath at 40°C for 10 minutes. The prepared solutions were filtered and analyzed under the GC-MS conditions described above.

The chromatograms and mass spectra obtained as a result of the analysis were compared with the database available on the computer. It was found during the analysis that the retention times and mass spectra of the fragment ions observed in the samples under investigation fully corresponded to the values characteristic of mephedrone and methamphetamine. This made it possible to reliably confirm that the substance being analyzed was mephedrone and methamphetamine. These results are shown in Figures 3-4.

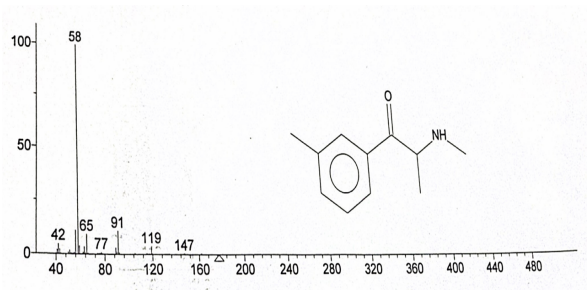


Figure 3. Mass spectra of the physical evidence.

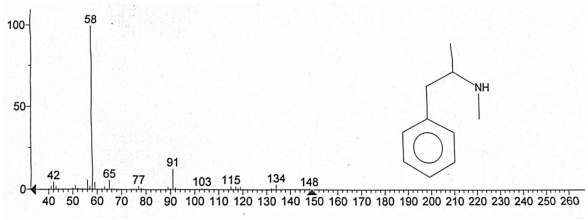


Figure 4. Mass spectra of the physical evidence.

To develop a method for isolating amphetamines from biological fluids, a method of extracting mephedrone and methamphetamine from an aqueous medium was used.

For analysis, 10 ml urine and 5 ml blood samples were taken, and 5 ml of a solution containing 1 mg/ml mephedrone and methamphetamine was added to them, and left for 2 hours. Then, a buffer solution prepared using a fixanal solution with a pH of 4.01 was added to the model samples, and the pH was adjusted to 4.0. Then, 2 ml of a 25% solution of ammonium sulfate and 10 ml of ethyl acetate were added to the mixtures, and shaken on a mechanical shaker for 10 minutes. After that, the mixture was centrifuged (3000 rpm) for 5 minutes to precipitate proteins. The organic layer was separated from the aqueous layer, and the remaining aqueous layer was extracted twice more with 10 ml of ethyl acetate, and the organic layer was poured off. The ethyl acetate extracts were combined, passed through filter paper containing 5 g of anhydrous sodium sulfate salt and previously moistened with the solvent, and the filtrate was evaporated until a dry residue remained. The dry residue was dissolved in 5 ml of 96% ethyl alcohol, and 1 ml was taken from it and dripped onto the starting line of the chromatographic plate in order to purify it from foreign substances by thin-layer chromatography (TLC). Then it was chromatographed in a chamber containing a mixture of ethyl alcohol-25% ammonia solution (100:1.5), the sorbent in the area where the substance was located on the plate was scraped off and eluted with 2 ml of ethyl alcohol. The eluate was filtered into a 10 ml volumetric flask and brought to the mark with 96% ethyl alcohol. The chromatographically purified isolate was analyzed by GC-MS using the method described above.

At the next stage of our experiment, a method for isolating mephedrone and methamphetamine from a biological object was developed. For this, samples of 50 g of animal liver were prepared, crushed, and 5 ml of a solution containing 1 mg/ml of mephedrone and methamphetamine was added and mixed well. The model objects were left at room temperature for 24 hours. Then, 0.02 M sulfuric acid solution was added to the model objects until a glass layer was formed, the pH was adjusted to 2-2.5 with a 20% solution of sulfuric acid, and left for 2 hours. After the specified time, the liquid was poured off from the object, and the object was again soaked twice with 0.02 M sulfuric acid solution for 2 hours and 1 hour. The resulting extracts were pooled. The pH of the pooled extracts was adjusted to pH=4 using a 25% solution of ammonium hydroxide and centrifuged at 3000 rpm. The centrifugates were poured off, and the residue was discarded. The centrifugates were saturated with ammonium sulfate salt, left for 1 hour, and centrifuged again. The centrifugates were shaken with 20 ml of ethyl acetate for 5 minutes, allowed to stand for 15 minutes, extracted, and the resulting extracts were filtered through filter paper containing 5 g of desiccated sodium sulfate salt and previously moistened with the solvent. The filtrate was left at room temperature until a dry residue was formed. The dry residue was purified by the TLC method shown in the analysis of urine and blood and analyzed by the GC-MS method described above.

The peaks and mass spectra of the chromatograms of the samples under study were identified by comparing them with the data in the computer base library.

The peaks of the chromatogram and mass spectra of the samples under study were compared with the database library called NIST2011.L, Wiley275.L, SWDRUG.L, CAYMANSPECTRA.L, PMW_TOX3.L (Figures 5-10)..

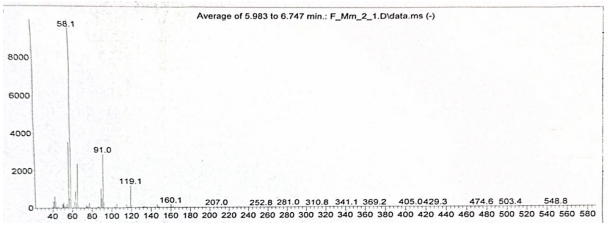


Figure 5. Mass spectra of mephedrone isolated from urine.

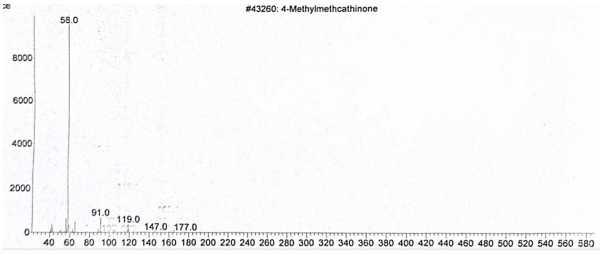


Figure 6. Mass spectra of mephedrone isolated from blood.

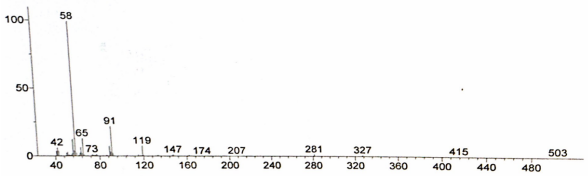


Figure 7. Mass spectra of mephedrone isolated from the model object.

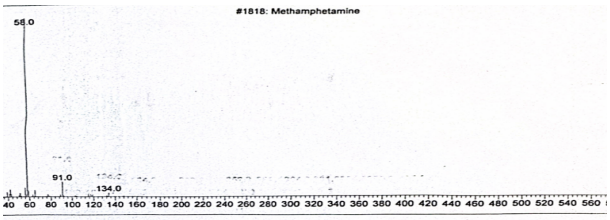


Figure 8. Mass spectra of methamphetamine isolated from urine.

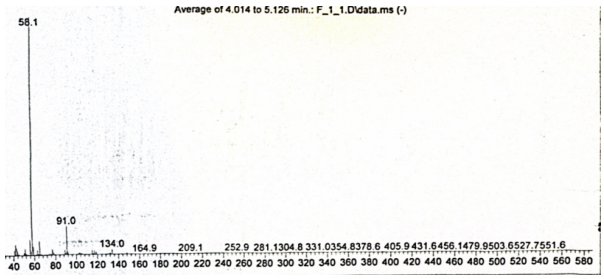


Figure 9. Mass spectra of methamphetamine isolated from blood.

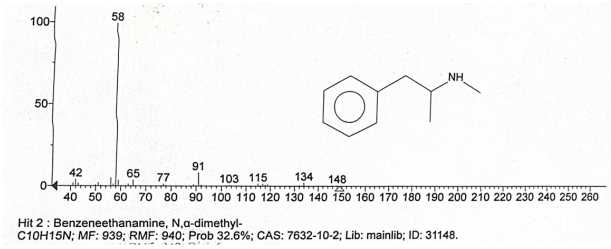


Figure 10. Mass spectra of methamphetamine isolated from the model object.

The data above indicate that the concentration of methamphetamine is higher than that of mephedrone in all biological samples, which suggests its greater stability and accumulation properties.

Conclusion and Discussion

As a result of the conducted research work, a method for the analysis of synthetic psychostimulants – mephedrone and methamphetamine – using the GC-MS method was developed for identification and quantification. Using the developed method, substances isolated from blood, urine, and liver were analyzed. The chromatographic retention time and fragment ions in the mass spectrum of each substance were determined and compared with international databases, in particular NIST (National Institute of Standards and Technology) and other spectral libraries. The analysis results showed a high degree of agreement with the structural features of the substances.

The conducted studies demonstrated the possibility of using the GC-MS method for the detection of mephedrone and methamphetamine in biological objects (blood, urine, liver)

During the research, the retention times of the peaks in the chromatogram of the standard sample of mephedrone and methamphetamine were 5.84 min and 3.69 min, respectively. Also, mass spectrometric fragment ions were determined for each substance, and their main peaks were as follows:

Main fragments for mephedrone: m/z = 65, 91, 119.

Main fragments for methamphetamine: m/z = 148, 91, 65.

Under the same conditions, the results obtained in the analysis of the confiscated physical evidence - mephedrone and methamphetamine powder - were found to correspond to their standard samples.

When the results obtained in the analysis of model objects were compared with the standard samples of the substances, the presence of mass spectra and ion fragments characteristic of mephedrone and methamphetamine was found.

The results obtained showed that the developed GC-MS method for the detection of mephedrone and methamphetamine in biological objects is highly selective, sensitive, and rapid. The possibility of detecting small amounts of amphetamines and their metabolites using this method is of current importance for forensic chemical examination.

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