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DEVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHODS FOR THE ANALYSIS OF MEPHEDRONE AND METHAMPHETAMINE

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Annotation: This article presents the development of a method for determining mephedrone and methamphetamine using high-performance liquid chromatography (HPLC). Research was conducted to develop an HPLC method for analyzing mephedrone and methamphetamine, which are included in the list of prohibited narcotic substances in the Republic of Uzbekistan. A method for extracting and identifying mephedrone and methamphetamine from physical evidence was developed. The linearity of the method was determined.

Under the selected analytical conditions of the HPLC method, the retention time of mephedrone was determined to be 5.158 minutes, while the retention time of methamphetamine was 4.320 minutes. This analytical method was confirmed to be specific for mephedrone and methamphetamine.

Keywords: amphetamines, mephedrone, methamphetamine, high-performance liquid chromatography, linearity, standard sample, retention time, chromatographic peak.

Relevance: Over the past decade, cases of poisoning with chemical substances have been increasing worldwide. Experts speak of a "toxic situation" developing in modern society. There has been a rise in the illegal use of narcotic drugs (ND), psychotropic substances (PS), and pharmaceuticals (P) [1].

A key feature of today's global drug market is the expansion of the range of illicit substances. Various pharmaceutical drugs with intoxicating effects at doses exceeding therapeutic levels are entering the market. Additionally, the proportion of illegally manufactured narcotic drugs with high concentrations of active ingredients is increasing, contributing to the intensification of illicit drug trafficking [2].

The fight against narcotics has become a major global concern, affecting not just individual families but entire societies. Currently, approximately 275 million people worldwide regularly use drugs—a 22% increase compared to 2010 [3].

Amphetamines pose several serious challenges to the international community. These substances are among the most widespread synthetic drugs found in illicit circulation [4].

In recent years, the volume of confiscated amphetamines has significantly increased in Western Europe, CIS countries, and Uzbekistan. The illegal drug market in Uzbekistan

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continues to grow and transform, adopting new forms. Illegally produced amphetamine analogs are often not tested for pharmacological activity, making their consumption highly risky. Overdoses or severe side effects may occur. Recently, several analogs of mephedrone, including modifications of the benzene ring, have been found to have toxic effects on the human body [5].

Objective of the Study: to develop methods for analyzing mephedrone and methamphetamine using high-performance liquid chromatography (HPLC).

Materials and Methods: the analysis of standard samples of mephedrone and methamphetamine using the HPLC method was conducted on an Agilent Technologies 1260 LC-20 Prominence liquid chromatograph equipped with a diode array detector (DAD) SPD-M20A and an autosampler. The system included a high-pressure, four-channel gradient pump, a UV-spectrophotometric detector operating in the 190–360 nm range, a Rheodyne injector with a 20 µL loop, and a chromatographic column.

The experiments were conducted under the following conditions: Chromatographic column: Stainless steel, packed with Perfekt Sil 300 ODS C18 sorbent (particle size 5 µm), 150 × 4.6 mm; Mobile phase: Acetonitrile-buffer solution (50:50); Flow rate: 0.500 mL/min; Injection volume: 20 µL; Detector wavelengths: 265 nm for mephedrone and 256 nm for methamphetamine; Total analysis time: 15 minutes.

Sample Preparation: Samples of 5, 10, 15, 20, and 25 mg of mephedrone and methamphetamine standard substances were separately weighed and placed in 100 mL volumetric flasks. Then, 20 mL of acetonitrile was added, and the solutions were sonicated at 60°C for 15 minutes. After cooling to room temperature, the volume was adjusted to the mark with solvent (Solution A).

From Solution A, a 5.0 mL aliquot was taken using a volumetric pipette and transferred to a 50.0 mL volumetric flask, and the volume was adjusted with acetonitrile. The solution was filtered through a 0.45 µm membrane filter before chromatographic analysis. The flow rate was set to 0.500 mL/min, and 20 µL of the sample was injected into the system.

Results: the retention times of mephedrone solutions under these conditions were as follows: 5mg/ml solution: 5.158 min; 10 mg/ml solution: 5.158 min; 15 mg/ml solution: 5.168 min 20mg/mL solution: 5.165 min; 25 mg/mL solution: 5.161 min The symmetry factor of the chromatographic peak was determined to be 0.95 (Figure 1).

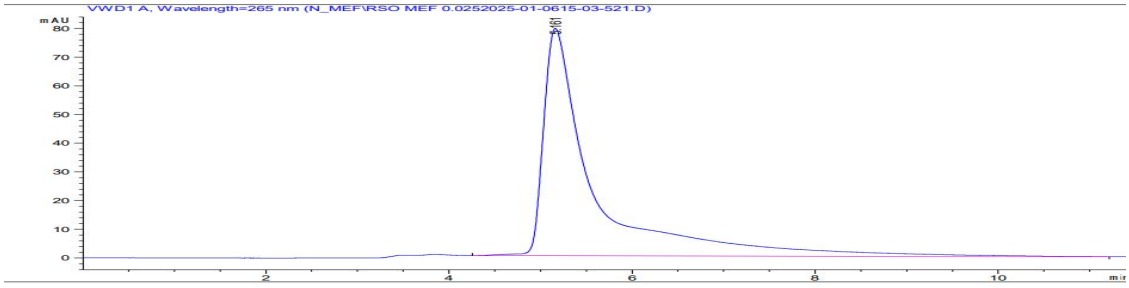

Figure 1. Chromatogram of the Working Standard Solution of Mephedrone

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At the next stage of the experiments, the linearity of this analytical method was studied. For this purpose, a standard sample containing mephedrone and extract solutions obtained from the test bio-object were used.

A 20 ml volume of the working standard and test solutions was injected into the chromatographic column under the previously mentioned conditions, and the corresponding chromatographic peak parameters were recorded.

Based on the obtained results, a calibration curve was plotted to determine the relationship between mephedrone concentration and peak area (Table 1 and Figure 2).

Table 1

Results of the Linearity Study of HPLC Analysis Conditions for Mephedrone

№	Substance Concentration in Solution (mg/ml)	Chromatographic Peak Area of Standard Substance	Retention Time (minutes)
1	5	704.045	5,158
2	10	985.559	5,158
3	15	1025.390	5,168
4	20	2239.015	5,165
5	25	3002.378	5,161

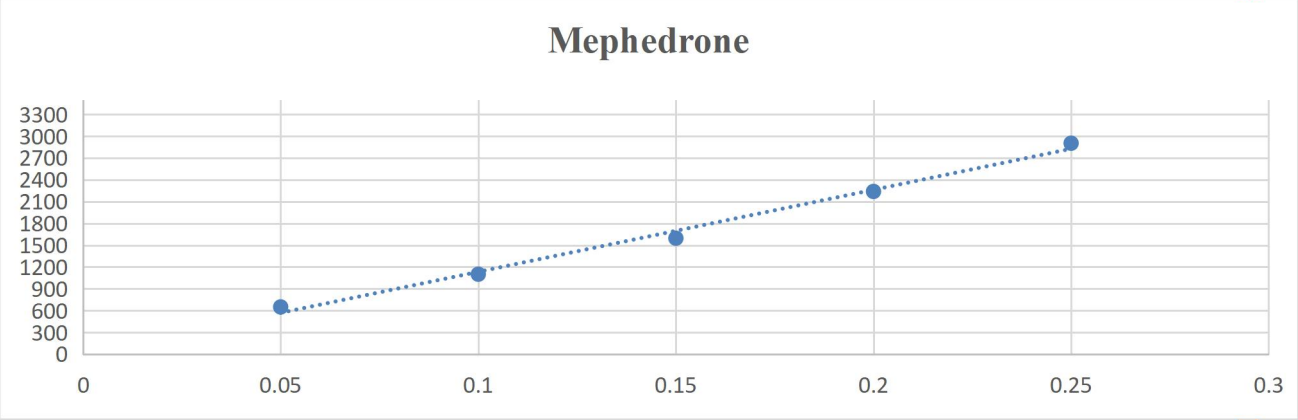

Figure 2. Calibration Curve of Mephedrone Peak Area vs. Its Concentration in Solution

Similar studies were conducted to develop an HPLC analysis method for methamphetamine. Standard samples were used to analyze methamphetamine with the developed method.

When methamphetamine was chromatographed using this method, a peak with a retention time of 4.320 minutes was obtained (Figure 3).

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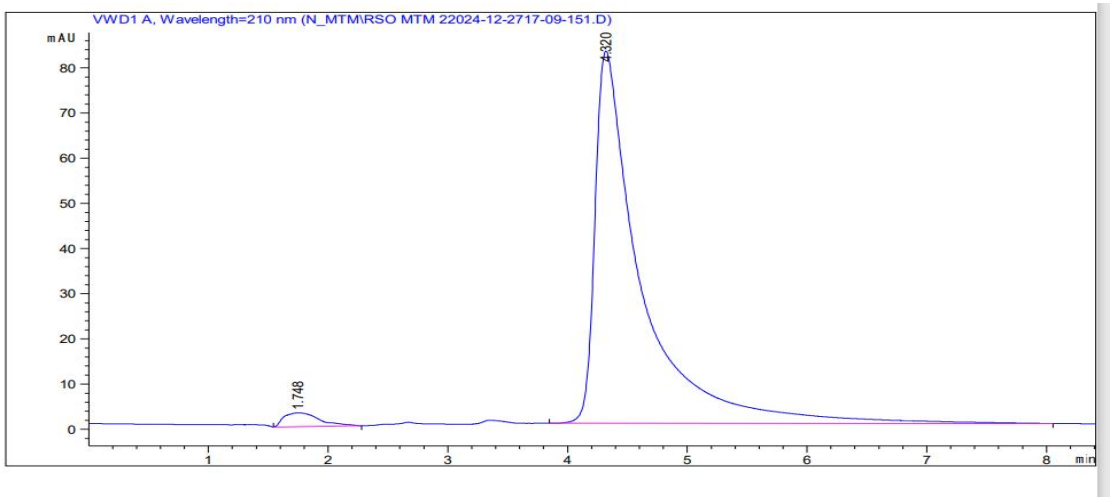

Figure 3. Chromatogram of the Working Standard Sample of Methamphetamine

To study the linearity of the analytical method for methamphetamine, standard sample solutions containing the substance were used.

A 20 µL volume of the working standard solutions was injected into the chromatographic column under the specified conditions, and the corresponding chromatographic peak parameters were recorded.

Based on the obtained results, a calibration curve was plotted to determine the relationship between methamphetamine concentration and peak area (Table 2 and Figure 4).

Table 2

Results of the Linearity Study of HPLC Analysis Conditions for Methamphetamine

№	Substance Concentration in Solution (mg/ml)	Chromatographic Peak Area of Standard Substance	Retention Time (minutes)
1	5	265.697	4,320
2	10	583.255	4,320
3	15	1148.271	4,380
4	20	2215.014	4,415
5	25	2403.395	4,439

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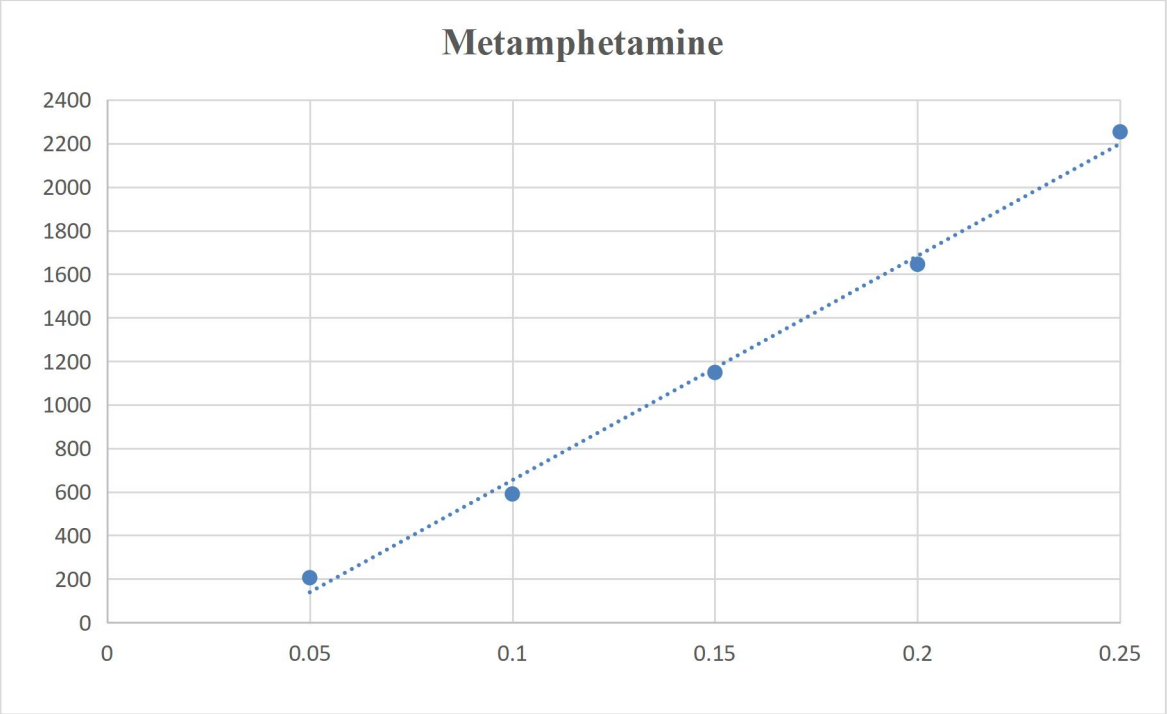

Figure 4. Graph of Methamphetamine Peak Area as a Function of Its Concentration in Solution

Conclusion

Under the specified conditions, the retention times for mephedrone solutions were as follows: 5 mg/ml solution → 5.158 min; 10 mg/ml solution → 5.158 min 15 mg/ml solution → 5.168 min; 20 mg/ml solution → 5.165 min; 25 mg/ml solution → 5.161 min. The chromatographic peak symmetry for mephedrone was determined to be 0.95.

Similarly, under the same conditions, the retention times for methamphetamine solutions were as follows: 5 mg/ml solution → 4.320 min; 10 mg/ml solution → 4.320 min; 15 mg/ml solution → 4.380 min; 20 mg/ml solution → 4.415 min; 25 mg/ml solution → 4.439 min. The chromatographic peak symmetry for methamphetamine was also determined to be 0.95.

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