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APPLICATION OF THE METHOD OF THERMAL-SECURITY SURFACE-IONIZATION SPECTROSCOPY IN ANALYSIS OF ALKALOIDS OF CONIUM MACULATUM

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ABSTRACT

Cicuta maculata is a species of flowering plants in the carrot family, known by several common names, including spotted water hemlock, spotted parsley, spotted cowboy and the root of suicide in the Iroquois. In this article authors studied efficiency of a new methodology in order to analyze the thermal desorption surface ionization spectroscopy of coniine and colchicine isolated from plant materials, using hemlock and biological fluids in the study of raw materials.

KEYWORDS: hemlock; thermal desorption surface ionization spectroscopy; colchicine; coniine.

INTRODUCTION

Hemlock spotted (Conium maculatum L.) is a widespread plant in Central Asia. Cases of poisoning give this plant a certain toxicological significance. In many cases, poisoning is found among the population and children who carelessly take the plant for parsley. In some cases, hemlock grains are taken as anise kernels, resulting in poisoning of a person. The main active substance of hemlock is that coniine is highly soluble in water and is rapidly absorbed into the body, affecting the central and vegetative nervous systems. In this regard, the issues of isolation and detection of hemlock alkaloids in biological fluids are of particular interest for chemical toxicological studies. The most toxicologically significant alkaloids of this plant are coniine, conhydrin and colchicine. The detection of hemlock alkaloids is carried out by chemical and physicochemical methods. However, the method of analysis of alkaloids of this plant by the method of thermal desorption surface ionization spectroscopy (TDSIS) [2] has not yet been developed.

The purpose of the study is to develop a methodology for analyzing the TDSIS of coniine and colchicine isolated from plant materials, using hemlock and biological fluids in the study of raw materials.

Experimental Part: For the detection of the main poisonous hemlock alkaloids - coniine and colchicine using the TDSIS method, the following analysis conditions were selected: emitter - oxidized molybdenum, incorporating iridium; emitter voltage - 405 V; emitter temperature –200-300°C; evaporation temperature - 20-505°C; air flow - 50 l/h (compressor voltage 12 V); the volume of the test - 0.1 ml; analysis time - 3 min; Spectra recording is performed directly using a computer program.

For research, solutions of standard samples of the studied alkaloids were prepared. Thermal desorption surface ionization spectra of coniine and colchicine were obtained using these solutions.

Then a series of solutions containing various amounts of alkaloids was prepared. The thermal desorption spectra of alkaloids and the values of the current strength corresponding to these spectra are shown in Fig. 1 and in Tables 1 and 2.

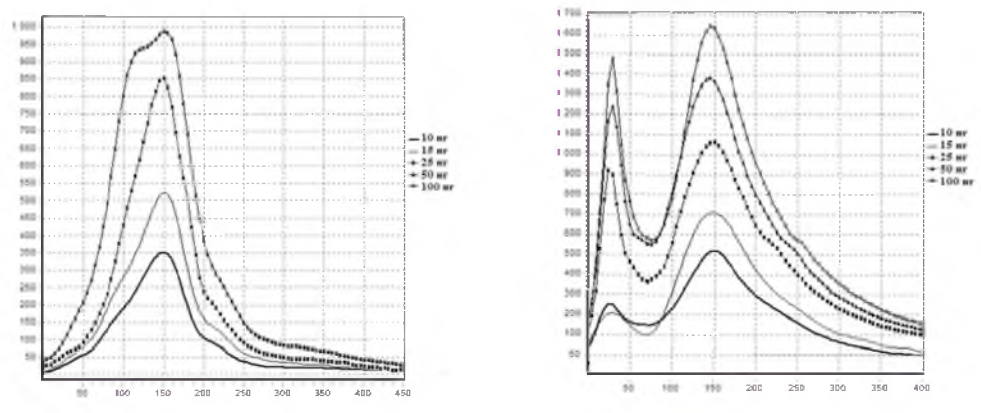


Fig. 1. Spectra of coniine (1) and colchicine (2).

Table. 1. The dependence of current strength TDSIS from the content of alkaloids in solution.

№	The content of coniine, ng	Amperage I, A
1.	10	400
2.	15	575
3.	25	900
4.	50	1049
5.	100	2500

№	The content of colchicine, ng	Amperage I, A
1.	10	352
2.	20	607
3.	30	1029
4.	50	1337
5.	100	1582

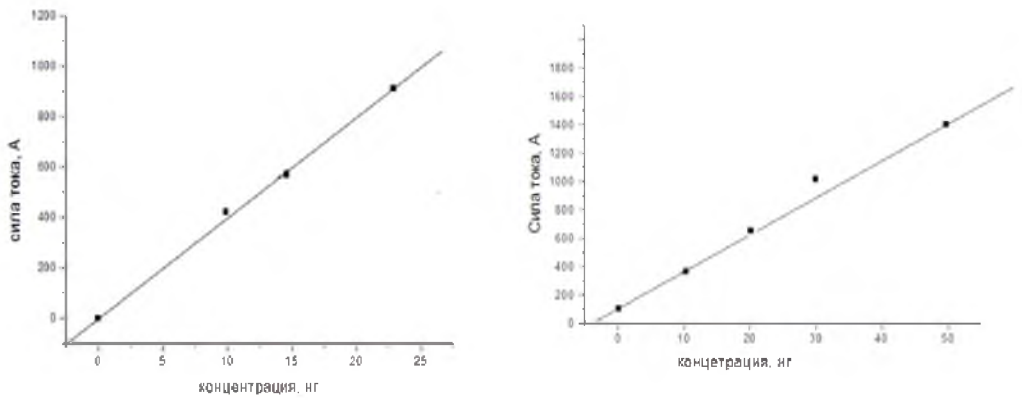


Fig. 2. Calibration graph for the quantitative determination of hemlock alkaloids (1-coniine, 2-colchicine).

On the basis of the obtained data, calibration graphs were constructed for the dependence of the current TDSIS of the spectra of coniine and colchicine on their concentration in solution (Fig. 2).

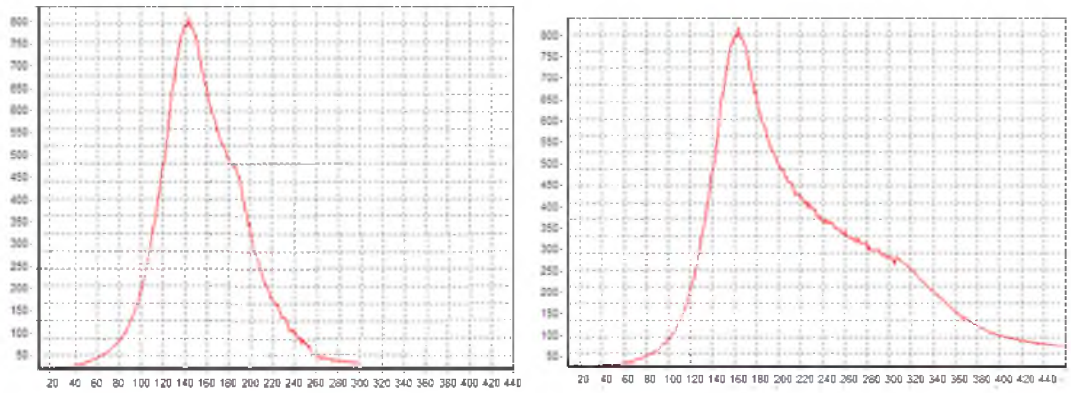
At the next stage of research, the developed methodology was tested in the analysis of coniine and colchicine isolated from plant materials and biological liquids (blood and urine). To isolate alkaloids from plant materials and from biofluids, an extraction method was used.

Extraction of alkaloids from raw materials of celandine

The raw material sample was ground to a particle size passing through a sieve with a hole diameter of 1 mm. About 10 g of crushed raw materials were placed in a 250 ml flask, 150 ml of diethyl ether and 7 ml of ammonia solution were added and the mixture was shaken for 1 hour. The ether extract was quickly filtered through cotton wool into a 200 ml flask, covering the funnel with a watch glass. 5 ml of water was added to the extract, it was vigorously stirred and left until the ether layer was clarified, after which the ether extract was transferred to a separatory funnel with a capacity of 200 ml. From the ether extract, the alkaloids were re-extracted as much as possible with 1% solution of hydrochloric acid in 20, 15, 10 ml portions (sample with Mayer's reagent), each time filtering through a 5 cm diameter paper filter

moistened with water into a second separating funnel of the same capacity. The filter was washed twice with a 1% 5 ml solution of hydrochloric acid, attaching the washing liquid to the total acidic extract.

The acidic extract was made alkaline with ammonia solution until alkaline (pH = 9) on phenolphthalein, and the alkaloids were extracted sequentially with 20, 15, 10 ml of chloroform, shaking for 3 minutes. The chloroform extracts were filtered into a distillation flask with a capacity of 100 ml through a filter paper, on which 4-5 g of freshly calcined anhydrous sodium sulfate moistened with chloroform were previously placed. The filter was washed twice with chloroform in 5 ml each. Chloroform was distilled off in a water bath to a volume of 1-2 ml, the rest of chloroform in the flask was removed by air flow until the smell of the solvent disappeared. The dry residue was dissolved in 5 µl of ethanol. Purification and, accordingly, separation of alkaloids was carried out by thin layer chromatography on Sorbfil plates, using methanol-concentrated ammonium hydroxide (100: 1.5) as the mobile phase. After treatment of the chromatogram with the acid iodoplatinate reagent, red-orange spots belonging to the coniine alkaloids (Rf=0.26), colchicine (Rf=0.33) and weakly orange color conhydrin (Rf=0.49) appear. Alkaloids coniine, colchicine and conhydrin were eluted by mixture of chloroform-methanol (95:5) solvents and evaporated to dryness at room temperature. The dry residue was dissolved in 2 ml of alcohol. 1 µl of the alcohol solution was injected into the cylindrical cavity of the vapor-forming tape of the PII-N-S Iskovich-1 apparatus and thermal desorption surface-ionization spectra were obtained. With the temperature range of 140-150 ° C and 150-160 ° C, the appearance of peaks characteristic of chelidonin and sanguinarine, respectively, was observed (Fig. 3).



1 2

Fig. 3. Spectra of coniinehead (1) and colchicine (2) isolated from hemlock grass Isolation of alkaloids from blood and urine.

Isolation from blood. 10 ml of 1 mol/l sodium hydroxide solution and 5 ml of chloroform were added to 5 ml of a model sample (cadaveric blood containing 50 μg of alkaloid isolated from a hemlock). The sample was shaken for 10 minutes and then centrifuged for 5 minutes at 3000 rpm. After separation of the layers, the lower chloroform layer was taken and passed through anhydrous sodium sulfate (0.5 g). Purification and separation of alkaloids was performed by Thin layer chromatography (TLC). The resulting eluates were evaporated in a stream of air to a volume of about 1 ml and transferred to a polypropylene tube, and then evaporated to dryness.

Isolation from urine. 10 ml of 1 mol/l sodium hydroxide solution and 5 ml of chloroform were added to 25 ml of a model sample (urine containing 50 μg of alkaloid isolated from a hemlock). The mixture was shaken for 10 minutes. After separation of the organic and aqueous layers, the lower chloroform layer was taken and passed through anhydrous sodium sulfate (0.5 g). Purification and separation of alkaloids was performed by TLC. The resulting eluates were evaporated in a stream of air to a volume of about 1 ml and transferred to a polypropylene tube, then evaporated to dryness.

The dry residue of extraction from blood was dissolved in 5 ml of alcohol. The dry residue extracted from urine was dissolved in 10 ml of alcohol. 1 μl of the resulting solution was injected into the cylindrical cavity of the vapor-forming tape of the FDI-H-S "Iskovich-1" apparatus and thermal desorption surface-ionization spectra were obtained. With the temperature range of 190–200°C and 194–209°C, the appearance of peaks characteristic of coniine and colchicine, respectively, was observed.

The results of the studies showed that in the analysis of alkaloids of hemlock spotted, isolated from bioliquids, the TDSIS method is suitable for both detection and quantitative determination of alkaloids. Using a calibration graph, the quantitative content of alkaloids extracted from model samples was calculated. The results obtained are statistically processed and are presented in table 3.

Table. 3: The results of quantitative determination of coniine and colchicine isolated from bioliquids.

The number of coniine		Statistical processing of Results	Amount of colchicine		Statistical processing of results
мкг	%		мкг	%	
Blood			**Blood**		
30,08 31,25 29,93 30,73 29,80	60,16 62,50 59,86 61,46 59,60	f=4; T (95%, 4)=5,84; X_{cp}=60,71; S^2=1,5058; S=1,2271; S_x=0,5487; Q_1=0,1034; Q_n=0,4482 E=11,8033%; ε=5,2786%	31,03 31,45 31,23 30,78 32,20	62,06 62,90 62,46 61,56 64,40	f=4; T (95%, 4)=5,84; X_{cp}=62,67; S^2=1,1734; S=1,0832; S_x=0,48; Q_1=0,1760; Q_n=0,5281 E=10,0936%; ε=4,5140%
Urine			**Urine**		
41,22 41,78 42,01 40,96 40,83	82,44 83,56 84,20 81,92 81,66	f=4; T (95%, 4)=5,84; X_{cp}=82,75; S^2=1,1828; S=1,0876; S_x=0,4863; Q_1=0,2047; Q_n=0,4409 E=7,6750%; ε=3,4324%	40,05 38,98 40,41 40,46 39,73	80,10 77,96 80,82 80,92 79,46	f=4; T (95%, 4)=5,84; X_{cp}=79,85; S^2=1,4681; S=1,2116; S_x=0,5418; Q_1=0,5067; Q_n=0,0337 E=8,8615%; ε=3,9629%

Findings: A technique has been developed for the detection of two alkaloids of a hemlock spotted by thermal desorption surface ionization spectroscopy. The possibility of using this method in the quantitative analysis of coniine and colchicine extracted from plant materials and bioliquids is shown. At the same time, coniine and colchicine is isolated from the blood in the amount of 60.71% and 62.67%, from the urine it is isolated in the amount of 82.75% and 79.85%, respectively.

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