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Elaboration of the Analysis Method of Ketamine Isolated from Biological Fluids Using Ultraviolet Spectrophotometry

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ABSTRACT: The article is devoted to the results of studies on the development of thin–layer chromatography (TLC) and UV–spectrophotometry analysis methods and introduction of ketamine to the determination of the substance extracted from the composition of biological fluids. The methods developed during the research were applied to the analysis of ketamine extracted from biological fluids and positive results were obtained.

KEYWORDS: thin layer chromatography, ketamine, extraction from biofluids (blood, urine), ultraviolet–spectrophotometry, thin–layer chromatography (TLC), poisoning.

INTRODUCTION

Phenylalkylamine derivatives mainly belong to the group of medicines affecting the central nervous system. One of the main representatives of this group is ketamine, which is used in medicine as a strong pain reliever and anesthetic. In recent years, the sharp increase in the sale of narcotic medicines and psychotropic substances, especially the widespread distribution of ketamine among young people, is the cause of severe poisoning and, as a result, deaths. In 2022, several cases of poisoning from this medicine were recorded in Tashkent and Samarkand regional branches of the Republican Scientific and Practical Center of Forensic Medical Expertise. In order to determine the cause of poisoning in these cases, physical evidence and internal organs of the corpse were submitted to the forensic chemistry department for chemical–toxicological examination. It should be noted that scientifically based chemical–toxicological testing methods of ketamine have not been developed.

In the literature, there is given information that the UV–spectrophotometry (UV–SP) method is widely used to determine the authenticity and amount of ketamine in medicines. However, in the studied literature, there is almost no information on the use of the UF–SP method in the analysis of ketamine extracted from biological fluids. Taking into account the above, it became clear that it is necessary to develop methods that meet the requirements of chemical-toxicological examinations of ketamine by chromatospectrophotometric method. In the process of extracting ketamine from the composition of biological fluids, co–extractive substances (unknown and ballast) pass into the extracts together with it. This helps in the identification and quantitative analysis of ketamine. Taking this into account, it is advisable to use the thin layer chromatography method to purify ketamine from foreign and ballast substances.

The aim of the study was to develop a method for the analysis of ketamine extracted from biological fluids by the chromatospectophotometry method.

Research methods and techniques. There were developed a thin–layer chromatographic analysis method for ketamine for the first time. The analyzes were carried out on Plaques CCM 20*20 cm Gel de silica 60 F254 of the German company MERK and ready plates (9.5x9.5 cm) “Sorbfil” manufactured in Russia.

It is known that standard sample solutions are used to identify and quantify the substance under investigation in Forensic Chemistry analyses. But because some medicines and substances are under special control, their standard samples are not always available in laboratories. Therefore, in such analyzes, various media containing these substances are used as “reliable” samples. Therefore, there was a need to prepare a “reliable sample” solution of ketamine–containing medicines. For this purpose, a “reliable

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sample" solution containing 100 μg/ml of the medicine containing 50 mg/ml ketamine was prepared. From this solution, "Fisherbrant" was transferred to a plate in a volume of 1 μl using a micropipette. The plate was chromatographed in chromatographic systems consisting of mixtures of chloroform and 95% ethyl alcohol (90:10) and chloroform and acetone (7:3). In order to determine the place where the substance rose and accumulated on the chromatographic plate, it was viewed using a UV–lamp irradiating at a wavelength of 254 nm, and a yellow–green precipitated spot was observed on the plate. Then Mune's modified Dragendorf's reagent followed by spraying with a 20% solution of sulfuric acid produced a dark purple spot on a white background.

In order to test the suitability of the conditions for the detection of ketamine in the next stage of the experiments, 0.5 mg of ketamine was separately added to 10 ml of blood and 10 ml of urine samples taken from the corpse as a model and left at room temperature for two hours. Then, using a universal indicator, the mixed medium was brought to pH 8.0–9.0 with a saturated solution of sodium bicarbonate. The mixtures were extracted 3 times with 10 ml of chloroform, the extracts were filtered through filter paper containing 0.5–1.0 g of anhydrous sodium sulfate. The filter paper was washed with 2–3 ml of chloroform and the wash was added to the main filtrate. The organic solvent was evaporated to a dry residue at room temperature. The dry residue was dissolved in 0.5 ml of alcohol and chromatographed under the above conditions. Then the plates were viewed under UV light, the borders of the formed spots were determined, and Dragendorf's reagent modified according to Mune, followed by spraying with a 20% solution of sulfuric acid. The results of the analysis are presented in table 1.

Table 1. Results of determination of ketamine by the *Thin–layer chromatography* (*TLC*) method

№	Selected solvent system	Biological fluids	
		in the blood	in the urine
1	Chloroform–95% ethyl alcohol (90:10)	0,60	0,62
2	Chloroform–acetone (7:3)	0,64	0,66

As can be seen from the results in Table 1, when the model analyzed ketamine isolated from blood and urine samples in the developed method, Rf=0.60 was obtained from the blood sample and Rf=0.62 in urine, and Rf=0.64 (blood) in the second chromatographic system. and produced dark purple spots with values of Rf=0.66 (urine). The results showed that the *TLC* method can be used to detect ketamine from the composition of biological fluids in case of ketamine poisoning.

Application of the UV–spectrophotometry method to the determination of ketamine. The UV–spectrophotometry method of analysis was carried out on a "SHIMADZU UV–1900" spectrophotometer. The optical density of the ketamine solution was measured at a wavelength of 200 to 400 nm. 95% ethyl alcohol was used as a reference solution. The optical densities of the prepared solutions were measured in the region of maximum light absorption of the substances, and a calibration plot was drawn using the obtained results. Based on the UV–spectra of solutions of different concentrations of reliable samples of ketamine, a calibration graph was drawn in the region of maximum light absorption.

As can be seen from the above researches, the dependence of the optical density of ketamine solutions on concentration is directly proportional in the range of 5.0–25.0 μg/ml, and concentrations in this range were found to obey the Bouguer–Lambert–Behr law. Therefore, the detection range also corresponds to this range of concentration values.

In the next stage of the experiments, the specific and molar absorption index of ketamine was calculated. For this, a series of working solutions with a concentration range of 5–25 μg/ml was prepared using the reliable sample solution dilution method and their optical density was measured. Based on the data obtained from experiments, the optimal value of the optical density of ketamine solutions is 0.505; set as 0.633. Based on these values, their specific and molar absorption index values were calculated. The result is presented in Table 2.

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Table 2. Relative and Molar Absorption Specimens of Ketamine

№	Amount of substance, μg/ml	Optical density, D	Comparative light absorption index, $E^{1\%}_{1см}$	Molar absorption index, Ɛ
1	5	0,111	22,2	527,6
2	10	0,221	22,1	525,3
3	15	0,370	24,6	586,1
4	20	0,505	25,2	600,1
5	25	0,633	25,3	601,8
			$E^{1\%}_{1см}$–23.88	Ɛ av 568,18

As can be seen from table 2, the average values of specific and molar absorption indices in the UV region were 23.88 and 568.18 values for ketamine, respectively. There were used to study the accuracy of the assay method 25 μg/ml solution of ketamine. The optical densities of ketamine solutions prepared at this concentration were measured in a UV–spectrophotometer at a wavelength of 269–276 nm, and the obtained results were metrologically processed. The results are presented in Table 3.

Table 3. The results of studying the accuracy of the developed analysis method

Ketamine amount, μg/ml	Determined		Metrological processing
	μg/ml	%	
25	24,9	99,6	$\overline{X}$ =99,98; T(95%–4)=2,78
25	24,8	99,2	S^2=0,8920; S=0,94445
25	25,0	100,0	Sx=0,4223Δ; $\overline{X}$ =1,1742
25	24,9	99,6	ΔX=2,6255; Ɛ=2,6261
25	24,7	98,8	$\overline{\varepsilon}$ =1,1744 %

As can be seen from the data given in table 3, when the results obtained by the UF–SP analysis method of ketamine were processed, its average relative error was 1.17%. This showed the developed method to be sufficiently accurate.

Model samples (blood and urine) were prepared in order to test the developed analysis methods at the next stage of experiments. For this, 10 ml of blood and 25 ml of urine were placed separately in clean 100 ml clean dry conical flasks. 5.0 ml of a stock solution containing 100 μg/ml ketamine was injected into it. Blood and urine samples were stored at room temperature for 24 hours, and then analyzed.

Isolation of ketamine from a model blood sample. There were added 10 ml of blood to a measuring flask with a capacity of 100 ml, pH was brought to 3.5–4.5 by dropping saturated solution of oxalic acid and left at room temperature for 2 hours. The mixture was then centrifuged at 3000 rpm for 10 minutes, the centrifuge was transferred to a separatory funnel, and the ballast was removed using 10 ml of ethyl acetate. The pH of the solution was adjusted to 7.5–8.0 using a 30% solution of sodium bicarbonate, controlled by a universal indicator. It was then extracted 3 times with 10 ml of chloroform. The chloroform extracts were combined and filtered through a filter paper containing 3–5 g of anhydrous sodium sulfate, previously moistened with chloroform, to remove moisture. The filtrate was dried at room temperature to a dry residue. The dry residue was dissolved in 1 mL of 95% ethyl alcohol and purified by TLC, and the eluates were collected. The eluates were dried and the residues dissolved in 95% ethyl alcohol and filtered through paper. The filtrate was analyzed by UV–spectrophotometric method.

The optical density of ketamine extracted from the model blood sample was 0.407 when measured at a wavelength of 269–276 nm.

Isolation of ketamine from a model urine sample. There were placed 25 ml of urine sample in a measuring flask with a capacity of 100 ml, pH of 3.5–4.5 was brought to it by dropping a 10% solution of sulfuric acid and left at room temperature for 2 hours. After that three times re–extracted with 10 ml of chloroform. The chloroform extracts were combined and filtered through a

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filter paper containing 3–5 g of anhydrous sodium sulfate, previously moistened with chloroform, to remove moisture. The filtrate is used to check its partial acidity. At the next stage, the aqueous part was brought to 7.5–8.0 using a 30% solution of sodium bicarbonate while checking the pH of the environment using a universal indicator, and was extracted 3 times with 10 ml of chloroform. The chloroform extracts were combined and filtered through filter paper with 3–5 g of anhydrous sodium sulfate, which had been previously moistened with chloroform, to remove moisture. The filtrate was dried at room temperature to a dry residue. The dry residue was dissolved in 5 ml of ethyl alcohol and filtered. A spectrophotometric analysis method was performed from the obtained filtrate.

The absorption index of ketamine extracted from the model urine sample was measured at a wavelength of 269 nm and the optical density was 0.452.

Determining the amount of ketamine in the tested biological fluids (μg/ml) was carried out based on the following formula:

$$x = \frac{D * V * 100}{\mathrm{E}\,{}^{1\%}_{1см} * \mathrm{a} * \mathrm{V1} * 100}$$

Here:
X–the amount of ketamine in micrograms/ml in the tested biological fluids;
D–the optical density of the solution;
E(1%)¦1cm–specific light absorption index of ketamine;
V_1 – the volume of the tested solution, ml;
V_2 – solution obtained for dilution, ml.
a–the starting sample of ketamine is 50 mg/ml.

Table 4. Results of ketamine extraction from biological fluid (blood, urine).

Determined amount of ketamine,%				Metrological processing	
blood		urine		blood	urine
μg	%	μg	%		
5,0	31,75	5,0	46,60	$\overline{X}$ =31,49; T(95%–4)=2,78	$\overline{X}$ =46,82; T (95%-4)=2,78;
5,0	31,68	5,0	46,55	S^2=0,3598; S=0,2585;	S^2=0,1932; S=0,43.46;
5,0	31,60	5,0	46,40	Sx=0,5664; ΔX=1,509;	Sx=0,1965; ΔX=2.5672;
5,0	31,30	5,0	47,45	$\Delta\overline{X}$ =0,6751; Ɛ=4,79%;	$\Delta\overline{X}$ =1.1412; Ɛ= 5,48%;
5,0	31,15	5,0	47,10	$\overline{\varepsilon}$ =2,14%;	$\overline{\varepsilon}$ =2,45%;

As can be seen from table 4, ketamine can be detected from the blood sample in an average amount of 31.49%, with a relative error of 2.14%, and from urine in an amount of 46.82%, with a relative error of 2.45%. In the process of extracting ketamine from biological fluids, significant losses were observed in the purification of foreign substances. According to the literature, this process directly depends on the metabolism of ketamine in the body, and different metabolites can be formed from the substance.

CONCLUSION

1. A method of thin layer chromatographic analysis of ketamine was developed. According to the results of the conducted studies, it was shown that in the case of ketamine poisoning, it is possible to clean the separations from the composition of biological fluids by the method of thin layer chromatography and carry out preliminary analysis methods.

2. The sensitivity and accuracy of the UV–spectrophotometry analysis method developed for the detection of ketamine was studied.

3. Methods developed for ketamine were tested on isolates obtained from biological fluids and positive results were obtained. This shows that the developed methods can be used in chemical–toxicological analysis.

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