AJLISI
GLOBAL RESEARCH NETWORK

Information Horizons: AMERICAN Journal of Library and Information Science Innovation
Volume 01, Issue 10, 2023 ISSN (E): 2993-2777

Development of Analytical Conditions for the Isolation of Ketamine from Urine by High-Performance Liquid Chromatography Method

Zakirdjan Abidovich Yuldashev
Doctor of Pharmaceutical Sciences, Professor, Tashkent Pharmaceutical Institute

Malokhat Ismatovna Nurmatova
Doctor of philosophy (PhD) pharmaceutical sciences, Tashkent Pharmaceutical Institute Tashkent, Republic of Uzbekistan

Abstract. *This article presents the results of the development of methods for extracting ketamine from the biological fluid urine and determining its amount using the method of high-performance liquid chromatography. The linearity and accuracy of the analysis method were studied. The possibility of detection and quantitative analysis of norketamine in extracts extracted from biological fluid using this method has been shown. In the practice of forensic chemistry, it is recommended to use the methods of chemical-toxicological analysis developed in cases of poisoning with ketamine.*

Keywords: *ketamine, urine, extraction, (HPLC), performance liquid chromatography.*

Introduction. Currently, there are many cases of acute and chronic poisoning with drugs and psychotropic substances, as well as cases of illegal abuse in the Republic of Uzbekistan. It is observed that the dependence of the representatives of the younger generation on certain drugs is increasing day by day [1]. These situations cause an increase in the number of deaths due to severe poisoning among young people. In clarifying the investigations opened in connection with these cases, physical evidence provided to forensic chemical departments by investigators or judicial investigation bodies, chemical toxicological tests of urine, blood samples and gastric juices taken from poisoned people or corpses that died as a result of acute poisoning of the urgent tasks for forensic chemists [2]. 85 types of psychotropic substances, whose circulation is restricted in the Republic of Uzbekistan, are among the highly effective poisonous drugs. Derivatives of the phenylalkylamine group included in this list are distinguished from other drugs by their sedative, analgesic effect on the central nervous system [3]. Ketamine, a representative of this group, is used in medicine as a strong pain reliever and anesthetic. In recent times, there has been a sharp increase in the use of ketamine for non-medical purposes, its sale on the black market and its widespread distribution among teenagers.

Acute poisoning from this drug was recorded. In this case, in order to determine the cause of poisoning, the physical evidence and the urine of the corpses were submitted to the forensic chemistry department for chemical and toxicological examination. Due to the insufficiently developed methods of scientifically based on chemical and toxicological examination of ketamine, problems arose in conducting the analysis. This showed that the development of methods of chemical-toxicological analysis of ketamine is one of the urgent tasks.

Purpose of work. Development of a method for extracting ketamine from urine and analysis by

high-performance liquid chromatography (HPLC).

Methods and techniques. Analyzes were carried out on a "SHIMADZU" LC-20 Prominence SPD-M20A chromatograph equipped with a UV detector. A column filled with stationary phase sorbent Perfekt Sil 300 ODS C-18 was used in the analyses. A column with a size of 150×4.6 mm was used in the analyses.

Chromatography conditions: UF-detector (detection zone set at 220 nm), as mobile phase: acetonitrile (solution A) and buffer solution (5.75 g of monobasic ammonium phosphate was dissolved in 1 liter of purified water and 6 ml of triethylamine was added in a volumetric flask with a capacity of 1 liter . The pH of the solution was adjusted to 3.0 using 85% phosphoric acid) (Solution B) was used. To carry out the analysis, the ratio of solutions A and B was 65:35, the flow rate was 1.0 ml/min, the injected volume was 20 µl, and the column temperature was set at 25°C. Chromatography time was 20 minutes. To develop the assay method, solutions of ketamine were prepared. Preparation of standard sample of ketamine. For this purpose, 2 ml of ketamine 50 mg/ml solution was placed in a 100 ml volumetric flask. 80 ml of 96% ethyl alcohol was poured into it. The mixture was shaken well and made up to the mark of the volumetric flask with 96% ethyl alcohol. Under these conditions, a stable solution of ketamine was chromatographed and its retention time was determined (Figure 1).

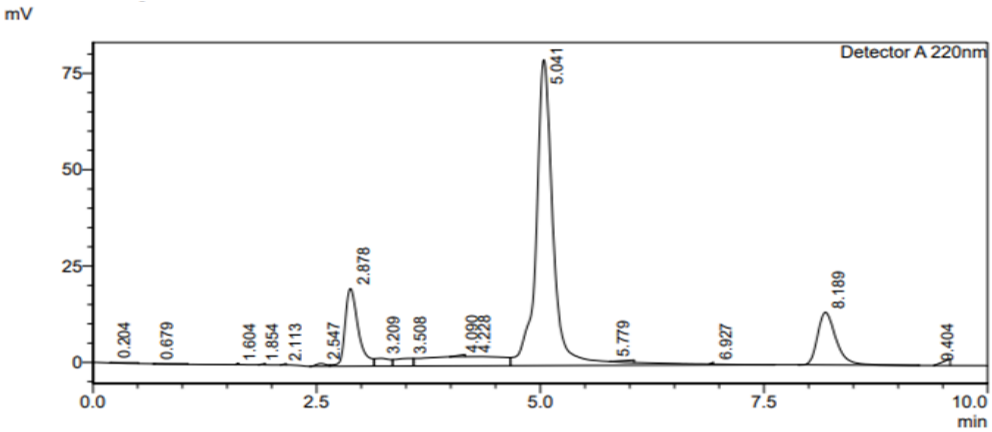

Figure 1. Chromatogram of a "Standard solution" of ketamine.

As can be seen from the chromatogram presented in Figure 1, a standard solution of ketamine hydrochloride produced a chromatographic peak at 5.041 min when chromatographed using the developed (HPLC) method. In the next stage of the experiments, a plot of the peak area of ketamine hydrochloride prepared from a standard solution was drawn as a function of its concentration in the solution, and the linearity of the analytical method was studied. For this, working solutions containing 5, 10, 15, 25 µg/ml of ketamine were prepared in the standard solution of ketamine. The solutions were chromatographed under the above conditions. The results are given in Table 1.

Table 1. The results of studying the linearity of the (HPLC) analysis method

The amount of substance in the solution, µg/ml	Chromatographic peak area surface of ketamine	Holding time, minute
5	128591	5,041
10	257182	5,042
15	385773	5,043
20	514364	5,044
25	642955	5,045

As can be seen from the data presented in Table 1, the retention time of the chromatographic peak heights of norketamine at different concentrations was shown in the range of 5.041-5.045 minutes. Chromatographic peak symmetry was found to be 0.95. In order to test the analytical

methods developed at the next stage of the experiments, a model biofluid sample of urine was prepared [4-5]. For this, 25 ml of urine sample was placed in a conical flask with a capacity of 100 ml, and 0.5 ml of the standard working solution of ketamine was added to it. The model urine sample was left at room temperature for 24 hours.Isolation of ketamine from a model urine sample. A 25 ml urine sample was placed in a dry clean flask with a capacity of 100 ml, a 10% solution of sulfuric acid was added dropwise to it, the pH of the medium was brought to 3.5-4.5 under the control of a universal indicator and it was left at room temperature for 2 hours. 3 was then re-extracted with 10 mL of chloroform. The chloroform extracts were combined, filtered through filter paper with 3-5 g of anhydrous sodium sulfate salt pre-moistened with chloroform to remove moisture and withdrawn for analysis of ketamine isolated in acidic medium[6]. The aqueous layer was brought to pH 9-10 with a 25% solution of ammonia and extracted three times with 10 ml of chloroform. The chloroform extracts were combined and crystallized at room temperature[7-8]. The dry residue was dissolved in 5 ml of 96% ethyl alcohol and analyzed by the (HPLC) method. The results are presented in Figure 2

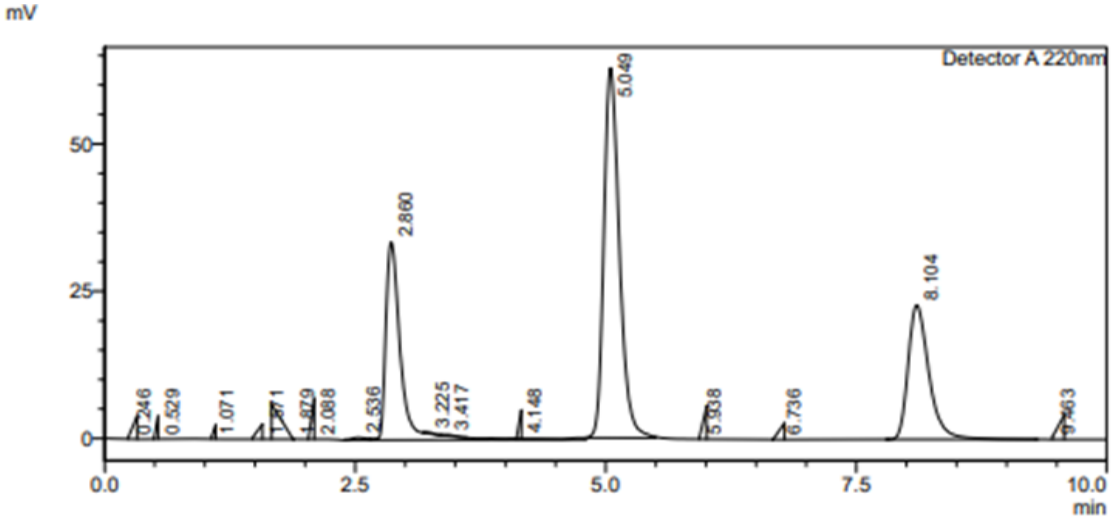

Figure 2. Chromatogram of ketamine isolated from urine

Extracts from the model urine sample produced a chromatographic peak at 5.049 min when analyzed by the developed method, which was shown to match the peak of a standard solution of ketamine. The presented chromatograms showed that the proposed method can be used in the identification and quantification of ketamine isolated from biological fluids, using the (HPLC) method. The next stage of the experiments was devoted to the study of the determination of the amount of ketamine extracted from biological fluids and the metrological analysis of the results. For this purpose, the biological fluid urine test isolate was purified by thin layer chromatography and measured five times to determine the amount of ketamine excreted. The results are presented in Table 2.

Table 2. Results of the extraction of ketamine from the biological fluid urine composition (added amount 0.5 mg)

The amount of ketamine detected		Statistical processing of the obtained results
мг	%	
0,316 0,314 0,316 0,307 0,313	63,20 62,80 63,20 61,40 62,60	f=4; T (95%,4)=2,78; $\overline{X}$ =62,64; S^2=0,5480; S=0,7402; S_x=0,3310; ΔX=2,0579; $\Delta\overline{X}$ =0,9203; Ɛ =3,28 %; $\overline{\varepsilon}$ =1,46%;

As can be seen from Table 2, ketamine can be identified from the urine sample in an average amount of 62.64% with a relative error of 1.46%.

Result. Ketamine standard working solution and extracts from biological fluids were identified by the (HPLC) method. In this case, the retention time of the chromatogram in the standard working solution of ketamine was 5.041 minutes, and the height of the chromatographic peak was 5.049 minutes in urine.

Conclusion: 1. A high-performance liquid chromatography analysis method for ketamine was developed, the sensitivity and accuracy of the method were studied. The suitability of the method for chemical-toxicological analysis was tested on the biological fluid urine and its practical application was demonstrated. 2. A graph of dependence of the surface of the chromatographic peak area of a reliable solution of ketamine on its concentration in the solution was made and a method of determining the amount of isolated ketamine was developed. 62.64% of ketamine was extracted from urine. 3. It was recommended to use the (HPLC) method developed for ketamine in forensic chemistry practice, to analyze cases of acute poisoning from this drug.

Literatures.

1. Salmi E., Langsjo J.W., Aalto S., et al. Subanesthetic ketamine does not affect 11C-flumazenil binding in humans. Anesth Analg. – 2005. – V. 101. – P. 722–725.
2. Seeman P, Ko K, Tallerico T. Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol Psychiatry. – 2005. – V.10. – P. 877-883.
3. Rizaev K. S., Yuldashev Z. A., Nurmatova M.I., Primukhamedova Kh. I., //Elaboration of the analysis method of ketamine isolated from biological fluids using ultraviolet spectrophotometry /. «International Journal of Current Science Research and Review». ISSN: 2581-8341. Volume 06 Issue 05 May 2023. DOI: 10.47191/ijcsrr/V6. Page no 15-19, Impact Factor: 6.789. IJCSRR @ Malayziya 2023.
4. Нурматова М.И., Юлдашев З.А. Обнаружение ацетамиприда в биологических объектах //IV международная научно-практическая конференция «Лекарства-человеку. Современные проблемы фармакотерапии и назначения лекарственных средств» Харьков, 2020. – С. 413-414.
5. Abidovich Y.Z., Ismatovna N.M. Development analysis methods of pesticides acetomipride isolated from a biological object by uv-spectrophotometry. Annals of the Romanian Society for Cell Biology, 2021, 25, 2, pp. 4067 - 40736.
6. Pulatova, D.K., Mamasoliev, A.I., Urmanova, F.F., Murotov, Sh.B. Study of free amino acids and elemental composition of common barley grains and its dry extract, 2023, Khimiya Rastitel'nogo Syr'ya, pp. 233-238.
7. Karieva E.S., Sadikova R.K., Nuridullaeva K.N. Perspectives of immortality (Helichrysum) in modern medicine and pharmacy. International Journal of Pharmaceutical Research, 2020, 12, 4, pp. 642 - 647
8. Turdieva Z.V., Mullajonova M.T., Azizov U.M., Mirakilova D.B.Q. Technologies for producing capsules “yuyuzin”. Indian Journal of Forensic Medicine and Toxicology, 2020, 14, 4, pp. 7660 - 7663