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STANDARDIZATION OF THE CONDITIONS FOR THE IDENTIFICATION OF GLICLAZIDE IN THE EXTRACTS OBTAINED FROM BIOLOGICAL OBJECTS

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The basis of treatment of type 2 diabetes mellitus form sulfonylurea derivatives such as glibenclamide, gliclazide and glimepiride. Gliclazide is the second generation drug which is produced in many countries under various by various trade names as mono-drug (Diaglizide, Diaglizide MR, Gliclazide MR, Diabeton MR) in tablets of 30, 60 and 80 mg and in the combination with metformin (Glimecomb, Dianorm-M) [2]. Considering the peculiarities of the usage of this medicine, it's toxicological hazard factors are: prevalence and tendency to increasing the type 2 diabetes mellitus, lifelong application, OTC availability, side effects, the risk of hypoglycemic states, the tendency of patients with this pathology to suicide attempts, etc. These factors cause the development of the side effects, which lead to the acute or lethal poisoning. Side effects of the toxic nature are developed with the doses that are higher than therapeutic, while the lethal poisoning is most frequently due to intentional (suicide) drug overdose with further development of hypoglycemia, cardiovascular events, and other pathological complications. According to the data of FDA and patientsville.com websites the number of reported cases of gliclazide poisoning in the period 2010-2014 were 545, including 61 lethal [3]. In accordance with the legislation of International judicial practice of poisoning of chemical substance for identification and determination of toxicant in the biological objects a forensic toxicology investigation should have been conducted. After isolation of toxicants from biological objects obtained extracts are contaminated with different impurities. Their presence in the extract will certainly influence on the chromatographic mobility of specified substances in a thin layer. In such cases, it is necessary to conduct TLC research in standardized conditions.

The aim of the work is standardization of the conditions for TLC identification of gliclazide in extracts obtained from biological objects by Stas-Otto method, which is general for chemical-toxicological analysis (CTA).

Materials and methods. Preparation of the examined solution of gliclazide (for isolation by Stas-Otto method): approximately 20.0 mg of standard sample of gliclazide was transferred into a 10 ml flask, added 3.0 ml of chloroform-methanol solution (1:1) and mixed. Preparation of the standard solution of gliclazide: approximately 10.0 mg (accurate weight) of gliclazide was transferred into 10 ml volumetric flask, dissolved in 5.0 ml of chloroform and completed to the mark with the same solvent. Preparation of the standard solution of caffeine: approximately 10.0 mg (accurate weight) of caffeine was transferred into 10 ml volumetric flask, dissolved in 5.0 ml of hot distilled water and completed to the mark with the same solvent. The modeling procedure of poisoning: 50 g of the chopped pig liver was transferred into a flask, added 3 ml of the chloroform-methanol solution of gliclazide (1:1). The mixture is diligently mixed and left for 24 hours at room temperature. The isolation of the examined drug from the liver tissue has been conducted with ethanol, acidified with oxalic acid according to techniques [1]. The obtained chloroform extracts further have been investigated by TLC. TLC conditions. Analysis has been performed on chromatographic plates Merck silica gel 60 F254 (Germany) and Sorbfil (Russian Federation), 10Ч10 cm. Before samples has been eluated, chromatographic plate has been previously washed with methanol and activated in heating oven at 110-120°C during half an hour. Dried chromatographic plate has been divided into three parts. 10 мl (1 мg/ml) of the standard solutions of gliclazide and caffeine has been plotted as a point on the start line of chromatographic plate in zone 1 and 2. j of gliclazide chloroform extract, obtained from liver tissue, has been applied as a 2 cm wide strip in zone 3. After chromatography in ethyl acetate the chromatographic plate dried and treated with 1% solution of vanillin or 5% solution of chloral hydrate (for gliclazide) and Dragendorff reagent (for caffeine).

Results: for the imitating of poisoning of selected biological object it has been saturated during a day with a solution of gliclazide in toxic concentrations. The isolation of toxicant from a liver tissue by Stas-Otto method has been conducted. According to the CTA methodology chloroform extract of gliclazide, obtained after isolation, for identification of toxicant in a thin layer has been examined. For the suitability checking of the used system, chromatography procedure of the gliclazide in the presence of the standard substance – caffeine has been performed. After chromatography of standard

sample caffeine, the color of its spots (brown) to the chromatographic plate and Rf values has been coincided with the literature sources, which indicated the suitability of the used chromatographic system. It was found that the processing of the second and third zones of chromatographic plate by reagents: 1% solution of vanillin or 5% solution of chloral hydrate in chromatographic plate has been visualized as individual spots with Rf value 0,46-0,47, which relevant the standard sample of gliclazide. Herewith, in the first case, the spot was dark blue, and the other – brown color.

Conclusion: standardization of the conditions for identification of gliclazide in extracts, obtained from biological objects by Stas-Otto method has been performed. It has been found that spots color and Rf values of gliclazide obtained by Stas-Otto method and Rf values of standard samples of gliclazide has been matched. The standardized conditions can be used for the toxicological investigations of biological objects for gliclazide poisoning.

Literature: 1. Карташов В.А., Чернова Л.В. Химико-токсикологический анализ: в 2 ч. / ч. 1. Выделение токсических веществ из биологических объектов. - Майкоп: ООО «Качество», 2008. - 188 с. 2. Pharmacological and pharmaceutical profile of Gliclazide: A Review / A. Sarkar, T. Tiwari, P. Bhasin, M. Mitra // J. Applied Pharmaceutical Science. – 2011. – Vol. 01(09). – P. 11-19. 3. Side_effects [Электронный ресурс]: – Режим доступа: http:// www.patientsville.com/medication/side_effects.