THE USE OF GAS CHROMATOGRAPHY FOR ANALYSIS OF CHLOROPYRAMINE IN BIOLOGICAL FLUIDS

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Monitoring of drugs in biological fluids in the study of the pharmacokinetics and pharmacodynamics, quantitative determination of drugs in extracts from biological material in conducting chemical-toxicological studies, require the development of analysis methods that are characterized by high sensitivity and selectivity. These methods belong chromatographic methods, including one of the most sensitive - a method of gas-liquid chromatography (GLC).

Chloropyramine hydrochloride (suprastin) – N-(2-pyridyl)-N-(chlorobenzyl)-N',N'-dimethyl ethylenediamine hydrochloride, antihistamine first generation, causes heavy toxicity at exceeding of doses, affects the nervous and respiratory systems, potentiates the toxic effects of drugs when used in conjunction with analgesics, psychotropic drugs and alcohol, with drug addiction. According to the structure of market of retail of antihistamines, suprastin (11.07%) took second place in Ukraine. Development of methods for its analysis in biological fluids is an actual task.

For extraction of the drug to the model mixture 10 ml of blood or urine containing 0,5 mg chloropyramine hydrochloride added 5 ml of 10% trichloroacetic acid solution, stirred and left for 2 hours with constant stirring at pH 2.0-2.5. The mixture was centrifuged at 3000-5000 rev/min for 10 min, the supernatant was poured and admixtures extracted with hexane with three portions of 5 ml. Hexane phases are not investigated.

The aqueous layer was basified with 0.1 M sodium hydroxide solution to pH 9.0-10.0 and chloropyramine-base extracted twice with chloroform portions of 10 ml, followed by centrifugation at 3000-5000 rev/min for 10 minutes. Chloroform extracts were combined and filtered through a filter paper («red tape») with 1.0 g of anhydrous sodium sulfate.

TLC-purification was performed in conditions - moving system solvents – ethylacetate-methanol-25% ammonia solution (85:10:5), chromatographic plate **S**orbfil – PTSH - AF - A, $Rf_{chloropyramine}$ = 0.60-0.63. Location reagents – UV-light and Dragendorff's reagent as modified by Mounier. At the level of spots of the standard solution of the chloropyramine with a part of the plate that was not treated with location reagents, removed a layer of sorbent area of 4,5 cm^2, transferred to a filter and twice eluted with portions of 5 ml of agent 0.1 M solution of hydrochloric acid and filtered through a filter («red tape»).

For identification and quantification of chloropyramine with GLC-chromatography was performed on a gas chromatograph «Agilent 19091 J-413» by: capillary column 50 m x 320 mm with a motionless liquid phase – 100% dimethylpolysiloxane, HP-1, 0,25 microns. The gas feed rate to the column: helium (mobile phase) – 60,0 ml/min; hydrogen – 40,0 ml/min. The drug was detected flame ionization detector. Linear programmed temperature column: 5 minutes at 180 °C; temperature increase from 180 to 200 °C at 15 °C / min and 200 to 255 °C at a speed of 6 °C/min; 6 min at 255 °C. Evaporator temperature was 250 °C, detector – 250 °C.

It was established that in conditions of analysis chloropyramine identified by retention time –16,08±0,2 min. (detection limit – 0,02 μg/ml) in the extracts. Quantitative determination of chloropyramine was performed by the absolute calibration of area peaks using the calibration graph. Interval of linear calibration curve was in the range of concentrations (0,1 – 2,0 μg/ml); limit of quantification of - 0,1-0,12 μg/ml.

According to the research found that when GLC assay in blood can be determined 41.3±4.21%, in urine – 59.8±3.42% chloropyramine.