# DETERMINATION OF CHLORPROMAZINE METABOLITE IN OBJECTS OF BIOLOGICAL ORIGIN BY CHROMATOGRAPHY

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**Introduction.** The growing use of neuroleptics leads to an increase in acute poisonings. According to statistics, the number of fatal overdoses is 18%. Such data make it necessary to search for new methods of diagnosis of poisoning by neuroleptics, in particular phenothiazines. Their main metabolite is S-oxides, but the issue of determining chlorpromazine by metabolites is currently not sufficiently developed.

**Aim.** The purpose of the research is to develop a technique for determining chlorpromazine S-oxide in urine by HPLC.

**Materials and methods.** Chlorpromazine S-oxide in urine was determined by HPLC. The research was carried out on a microcolumn liquid chromatograph Milichrome A-02 in the reversed-phase version on a sorbent with a grafted chemically non-polar phase - Nucleosil-100-5, C-18 (metal column size 2×75 mm). A UV spectrophotometer was used as a detector. Detection was done in the wavelength range of 190-360 nm. Automatic sampling was provided by autosampler. As an eluent, 0.2 M lithium perchlorate with 0.01 M phosphoric acid (pH 2.2) and acetonitrile was used, which was fed in a gradient mode 2-100% acetonitrile. The S-oxide was found by retention time.

**Results and discussions.** S-oxide was obtained by the method in collaboration with professor M.Ye.Blazheyevskiy. For this purpose, 0.7615 g (0.002 mol) of chlorpromazine hydrochloride was dissolved in 5 ml of water, while stirring, a solution of peroxyacetic acid (0.316 mol) was added to the red color and left at room temperature for 15 min. 4 ml of 0.19 M NaOH to pH 9 was added to the mixture. Extraction was done in separatory funnel with 50 ml of diethyl ether. The separated organic phases were combined and evaporated to a dry residue in air stream, which was dissolved in ethanol. The yield of S-oxide was 93%. The structure of the substance was found by melting temperature and PMR spectroscopy data. Modeling of poisoning was performed by saturating 10 ml of urine with 0.5 ml of ethyl solution of S-oxide with 20 μg of the substance. For isolation, 0.1 M HCl was added to 10 ml of the urine mixture to pH 2 and extracted three times with diethyl ether in 5 ml portions. The aqueous layer was alkalized with a 50% NaOH to pH 11 and extracted three times by chloroform in 10 ml portions. The chloroform extractions were combined and filtered through a red tape paper filter with 1 g of anhydrous sodium sulfate into a 25.0 ml flask, the volume was brought up to the mark with chloroform. The obtained extract was analyzed by HPLC. Chlorpromazine S-oxide was found by retention time (tR), 13.70 – 14.17 min. The lower limit of the determined concentration of S-oxide Cn is 0.002 μg in 2 μl of sample (n=5; P=0.95). The RSD of the average when determining the substance in a solution of 2 μg/ml does not exceed 2% (n=5; P=0.95).

**Conclusions.** The developed technique for determining chlorpromazine by the product of S-oxide metabolism by HPLC in urine can be used for analytical diagnosis of acute poisoning with this drug.