DETERMINATION OF CYPROHEPTADINE IN URINE BY CHROMATOGRAPHIC METHODS

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Introduction. Cyproheptadine hydrochloride (peritol) - 4-(5H-dibenzo[a,d] cyclohepten-5-ylidene) -1-methylpiperidine hydrochloride is an antihistaminic drug with an antiserotonin effect, which prevents development and facilitates the course of allergic reactions. Cyproheptadine is characterized by antipruritic, antiexudative, anticholinergic and sedative effects. Cyproheptadine can cause intoxication of the body and the lethal effects of overdosing, self-medication and in cases of suicide. The elaboration of highly sensitive and selective methods for the study of cyproheptadine, suitable for analysis in biological objects is an actual task. Analysis of cyproheptadine in biological matrices during treatment or after human death is based on the selection of highly sensitive and selective methods of their investigation.

Purpose of work – the development of the algorithm for direct urine analysis for cyproheptadine using modern methods of extraction, purification of extracts and quantitative determination.

Materials and methods of research. The urine models with cyproheptadine were used in the studies: to 10,0 ml of the corresponding biological fluid was added 500,0 μg of cyproheptadine hydrochloride using an aqueous solution of substance containing 1000,0 μg / ml, as well as control samples were left for 24 hours at room temperature.

Method of cyproheptadine extraction from urine and purification of extracts by TLC-method. To 10,0 ml of a model mixture of urine with cyproheptadine hydrochloride, 5,0 ml of a 10% solution of acid trichloroacetic was added, mixed and checked with a universal indicator pH of the mixture − 2,0-2,5, left for 2 hours at constant stirring. The mixture was centrifuged at 3000-5000 rpm for 10 min, the liquid over the precipitate was separated and the extracts were extracted with hexane in portions of 5,0 ml three times. Hexane phases were not investigated. The aqueous layer was alkalinized with a 0,1 M solution of sodium hydroxide to pH 9,0-9,5 and the cyproheptadine-base was extracted twice with chloroform in portions of 10,0 ml followed by centrifugation at 3000-5000 rpm for 10 min. Chloroform extracts were combined and filtered through a paper filter ("red tape") with 1,0 g sodium sulphate anhydrous. The solvent was evaporated at room temperature to a dry residue, which was dissolved in 2,0-3,0 ml of methanol. The resulting methanol solution was quantitatively transferred to volumetric flask of capacity 5,0 ml, and the volume of solution was brought to the mark with methanol, and examined by TLC-method.

TLC-purification of cyproheptadine in extracts was carried out according to the the conditions: system of mobile solvents - ethylacetate - methanol -25% solution of ammonium hydroxide (85:10:5); chromatographic plates - glass plates by "Merck" (Germany) (R_f = 0,50±0,02). It was found that the most sensitive location reagents for cyproheptadine are UV light, λ = 254 nm (the sensitivity - 0,5-1,0 μg in the sample); reagent of Dragendorff in the modification of Mounier (the sensitivity - 3,0-

5,0 μg in the sample).

Quantitative determination of cyproheptadine was carried out using the HPLC-method on microcolumn liquid chromatograph "Milichrome A-02" ("EcoNova" Novosibirsk, Russia) using standardized HPLC conditions: reversed-phase variant with using of metallic column with non-polar absorbent Prontosil 120-5C 18 AQ, 5 μm; mobile phase in the mode of linear gradient – from eluent A (5 % acetonitrile and 95% buffer solution - 0,2 M solution of lithium perchlorate in 0,005 M solution perchloric acid) to eluent B (100% acetonitrile) as during 40 min. Regeneration of column has been conducted during 2 min with mixture of solvents; the flow rate of the mobile phase has been formed 100 μl/min, injection volume – 4 μl. The detection of cyproheptadine has been conducted by UV-detector at 8 wavelengths: 210, 220, 230, 240, 250, 260, 280, 300 nm; the optimal value of column temperature – 40°C and pressure of pump – 4,2 MPa.

For quantitative HPLC determination of cyproheptadine by absolute calibration method using the calibration curve constructed in the coordinates: S, mm2 (peak area) – C, μg / ml (solution concentration of the substance). In applying the method of least squares regression coefficients were calculated corresponding equation S = вC + a. The proposal the calibration curve meets equation of the line that has the form: $S = 0{,}53 \cdot 10^{-3} C - 0{,}48 \cdot 10^{-3}$, where S - area of peak cyproheptadine, mm^2; C - concentration of the solution of cyproheptadine hydrochloride, μg / ml. The correlation coefficient was 0,9980. Established that the linearity of the calibration curve in coordinates (S, mm^2) - (C, μg / ml) was observed in the concentration range 10,0 – 200,0 μg / ml, which corresponds to cyproheptadine hydrochloride content in the sample (4 μl) of 40, 0 ng to 800,0 ng respectively. The limit of determination of cyproheptadine hydrochloride by HPLC method was 10,0 μg / ml, which corresponds to 40,0 ng of sample. In conducting HPLC analysis of cyproheptadine in model solutions using the proposed method relative uncertainty of the average results did not exceed ± 2,09%.

Results and discussion. The identification of cyproheptadine was conducted with using absolute parameters of retention time (t_R = 22,71 ± 0,04 min) and retention volume (V_R = 2269,9 ± 0,4 μl). Established that the values of coefficients peak symmetry - 1,01± 0,08 (less than 2,0 – 2,5) and the coefficients of capacity - 14,13± 0,03 (more than 0,5 – 2,0) showed the suitability of HPLC chromatographic analysis system. To ensure reliable detection of cyproheptadine were used spectral ratio values absorbance at wavelengths - from 220 to 300 nm - the values of absorbance at 210 nm, which are equal: 1,381±0,005; 1,271±0,005; 0,924±0,006; 0,665±0,007; 0,309±0,005; 0,0428±0,004; 0,366±0,006.

It was established that method of extraction of cyproheptadine from urine, the allows to allocate 64,42 – 68,76% substance ($\bar{\varepsilon}$ = ± 3,26%, $RSD_{\bar{x}}$ = 1,17%).

The results of quantitative HPLC determination of cyproheptadine in urine are intended for employees of the Bureau of Forensic Medical Examination, toxicological and narcological centers, clinical laboratories for the study of medicinal substances in biological objects.