ANALYSIS OF PHENCAROL BY HPLC METHOD

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Introduction. Phencarol - Quinuclidyl-3-diphenylcarbinol hydrochloride is the first-generation antihistamine drug, is characterized by moderate antiserotonin action, weak cholinoblocking activity, absence of oppression effect on the central nervous system. Phencarol is used to treat allergy, asthma, allergic rhinitis and dermatitis. When overdosing medication develop symptoms: hallucinations, incoordination, convulsions. In severe intoxication develops a coma with respiratory failure.

Among the modern methods of analysis to create a database of parameter identification and quantification of arrays of analytes in biological objects HPLC method is one of the most suitable methods for sensitivity and selectivity.

The earlier developed methodology of HPLC analysis of Phencarol using a variety of different chromatographic conditions (type of sorbent, composition of the eluent, the elution rate, detection conditions), which are based on the individual properties of the drug. Considering the use mixtures of drugs for the treatment and combined intoxications actual problem of chemical-toxicological analysis is the use of unified HPLC method suitable for solving practical problems of healthcare.

Aim. The identification and quantification of Phencarol, when using unified conditions HPLC, suitable for studies of pharmaceuticals and biological objects.

Materials and method. Investigations of Phencarol by HPLC-method were performed on the basis of scientific-production association "Analytics" (Kharkov). Chromatography of Phencarol was performed 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 Phencarol 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. To select the detection conditions Phencarol were obtained UV-spectrs absorption of drug solutions in solvent mixtures - 5% acetonitrile and 95% buffer solution when using SF-46

spectrophotometer, cuvettes thickness of 10 mm, in the range of 220-350 nm, reference solution - buffer solution.

Results and discussion. The identification of Phencarol conducted with using absolute parameters of retention time (t_R = 20,27 ± 0,10 min) and retention volume (V_R = 2027,1 ± 0,1 μl). To verify the choice chromatography conditions determined coefficients of peak symmetry and coefficients of capacity. Established that the values of coefficients peak symmetry - from 0,63 to 1,01 (less than 2,0 – 2,5) and the coefficients of capacity - from 12,51 to 12,62 (more than 0,5 – 2,0) showed the suitability of HPLC chromatographic analysis system.

To ensure reliable detection of Phencarol used spectral ratio values absorbance at wavelengths - from 220 to 280 nm - the values of absorbance at 210 nm, which are equal: 0,634; 0,255; 0,041; 0,022; 0,028; 0,001; 0,0003. The detection limit of Phencarol HPLC method was 5,0 μg / ml or 20,0 ng of sample.

For quantitative HPLC determination of Phencarol by absolute calibration method using the calibration curve constructed in the coordinates: S, mm^2 (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 = 0,00849 C + 0,00329, where S - area of peak drug, mm^2; C - concentration of the solution of Phencarol, μg / ml. Established that the linearity of the calibration curve in coordinates (S, mm^2) - (C, μg / ml) was observed in the concentration range 5,0 – 100,0 μg / ml, which corresponds to Phencarol content in the sample (4 μl) of 20, 0 ng to 400,0 ng respectively.

The limit of detection of Phencarol by HPLC method was 5,0 μg / ml, which corresponds to 20,0 ng of sample. In conducting HPLC analysis of Phencarol in sample solutions using the proposed method relative uncertainty of the average results did not exceed ± 1,87%. As a result of the metrological characteristics found no significant systematic errors HPLC analysis.

Conclusions. Identification and quantification of Phencarol by unified HPLC-conditions were conducted. The main parameters of retention, spectral relations and detection limit of the drug (20,0 ng of sample) were established.

As a result of the quantitative determination of Phencarol by HPLC method defined linearity range depending on the peak area and concentration -5,0 - 100,0 μg / ml of the drug and the limit of detection - 5,0 μg / ml. In carrying out HPLC-analysis of Phencarol in model solutions relative uncertainty of the average result equal ± 1,87%.