ANALYSIS OF TERAZOSIN BY HPLC METHOD

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Introduction. Terazosin hydrochloride – (RS)-6,7-dimethoxy-2-[4-(tetrahydrofuran-2-yl-carbonyl)-piperazin-1-yl]quinazolin-4-amine hydrochloride – like other quinazoline derivatives (doxazosin, alfuzosin and prazosin), belongs to the group of α_1-adrenoblockers and is used in medical practice for the treatment of arterial hypertension and prostatic hypertrophy. When applying terazosin, there are possible side effects: headache, weakness, dizziness, insomnia, due to active lowering of blood pressure. Destruction of the functions of the digestive tract is manifested by nausea, dry mouth, oral allergic reactions, peripheral edema. In case of overdose or self-medicate with terazosin the cardiovascular system is affected, the activity of the central nervous system is suppressed, respiratory system is broken [1,2].

The previously developed methods of HPLC analysis of terazosin hydrochloride are distinguished by the use of different chromatographic conditions, which are based on the individual properties of investigated substance. Method of identification and quantification of terazosin by HPLC method in the application of various detection options in various matrices was carried out using different sorbents, composition of moving phases, buffer solutions in isocratic and gradient elution modes [3,4]. Given that, with insufficient or slow development of the antihypertensive effect, terazosin is combined with diuretics, β-adrenoblockers or other antihypertensive agents, an important stage for the further research of medicinal substances is the development of a unified HPLC method and the creation of databases by the parameters of identification and quantitative determination of analytes. The results of research on a unified HPLC method can be recommended for the introduction into the practice of the bureau of forensic examination, toxicological centers, clinical laboratories regarding the study of medicinal substances in biological objects.

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

Materials and method. Investigations of terazosin by HPLC-method were performed on the basis of scientific-production association «Analytics» (Kharkov). Chromatography of terazosin 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 terazosin 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. The results of the identification and quantitative determination of the HPLC method were calculated using the computer program «MultiChrom» (Ampersend, Closed Joint-Stock Company, Moscow, Russia), which was part of the chromatograph.

Results and discussion. The identification of terazosin conducted with using absolute parameters of retention time (t_R = 14,41 ± 0,20 min) and retention volume (V_R = 1441,1 ± 0,2 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 0,78 to 1,09

(less than 2,0) and the coefficients of capacity – from 0,87 to 0,90 (more than 0,5) showed the suitability of HPLC chromatographic analysis system. To ensure reliable detection of terazosin used spectral ratio values absorbance at wavelengths – from 220 to 300 nm – the values of absorbance at 210 nm, which are equal: 0,816; 0,833; 1,657; 1,899; 1,118; 0,278; 0,111. The detection limit of terazosin HPLC method was 5,0 μg / ml or 20,0 ng of sample.

For quantitative HPLC determination of terazosin by absolute calibration method using the calibration curve constructed in the coordinates: S, mm^2 (peak area) – C, μg / ml (concentration of solution 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: $S = 4{,}4 \cdot 10^{-4}$ C – $5{,}1 \cdot 10^{-4}$, where S – area of peak drug, mm^2; C – concentration of solution of the substance, 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 terazosin content in the sample (4 μl) of 20, 0 ng to 400,0 ng respectively. The limit of detection of terazosin by HPLC method was 5,0 μg / ml, which corresponds to 20,0 ng of sample. In conducting HPLC analysis of terazosin in sample solutions using the proposed method relative uncertainty of the average results did not exceed ± 1,98 %. As a result of the metrological characteristics found no significant systematic errors HPLC analysis.

The accuracy and precision of the HPLC – technique of quantitative determination of terazosin were established by the values of the relative standard deviation of the average result in percentages for various concentrations of the test substance in model solutions using a calibration graph or equation of direct dependence. It was established that the accuracy and precision of the results of the application of the developed method for concentrations of terazosin in the interval of linearity of the calibration graphs in model solutions did not exceed 1,0, which indicated the proximity of the results of the analysis to their true value. In a comparative assessment of the reproducibility of terazosin analysis by HPLC, it was found that the values of the relative standard deviation of the results of terazosin analysis during one day (intra-day) and during the second day (inter-day) in the region of low (10,0 μg / ml), medium (40,0 μg / ml) and high concentrations (100,0 μg / ml) did not exceed 0,79.

Conclusions. Identification and quantification of terazosin 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. The HPLC method of determination of terazosin was validated by parameters: linearity range, quantification limit, accuracy and precision in the regions of low, medium and high concentrations of the substances. As a result of the quantitative determination of terazosin 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 terazosin in model solutions relative uncertainty of the average result equal ± 1,98 %.

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