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THEORETICAL AND PRACTICAL ASPECTS OF MODERN SCIENTIFIC RESEARCH

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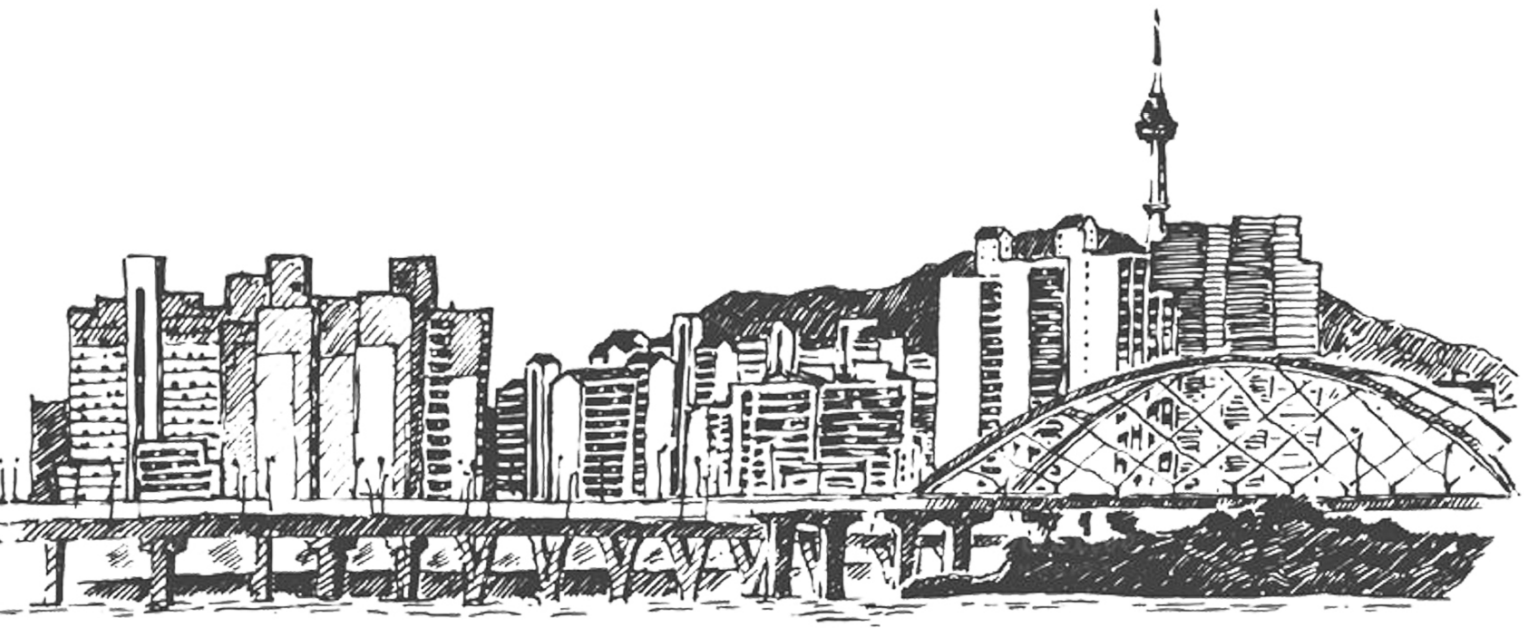

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THE DETERMINATION OF ATENOLOL BY HPLC METHOD

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Introduction. Atenolol - 4- [2 - Hydroxy – 3- [(1–methylethyl) amino] propoxy] benzene acetamide - β–adrenoceptor blocking agent is used to treat stenocardia, arterial hypertension, tachycardia, myocardial infarction [1,2]. Atenolol can cause bradycardia, hypotension, depression, hallucinations, allergies and as a result of overdose and self-treatment, it can cause intoxication of the organism and the lethal effects [3,4].

For therapeutic monitoring during the treatment of patients, for the study of biological objects on atenolol as a result of intoxication and lethal consequences, the use of highly sensitive, rapid and effective methods of analysis is important.

The most common chromatographic methods for analysis of atenolol are gas-liquid chromatography (GLC) [3], high performance liquid chromatography (HPLC) [3,5], gas chromatography–mass spectrometry (GC–MS) [6]. The previously developed methods of HPLC analysis of atenolol are distinguished by the use of different chromatographic conditions, which are based on the individual properties of investigated substance.

An important stage for the further research of medicinal substances and their mixtures in biological objects is the development of a unified HPLC method and the creation of databases by the parameters of identification and quantitative determination of analytes.

Purpose of work – the development of analysis of atenolol by unified HPLC method.

Materials and methods of research. Chromatographic analysis was carried out on a microcolumn liquid chromatograph "Milichrome A-02" (EkoNova, Closed Joint-Stock Company, Novosibirsk, Russia) according to the unified HPLC methodology developed by the author - Baram G.Y. 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 atenolol 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.

Results and discussion. The identification of atenolol was conducted with using absolute parameters of retention time, retention volume and spectral ratio (tables 1,2).

Table 1

Retention parameters of the atenolol by HPLC method (n = 5, *P = 95%)*

Parameter	Parameter values	Metrological characteristics					
		$\overline{X}$	S	$S_{\bar{x}}$	$\Delta_{\bar{x}}$	$RSD_{\bar{x}}$	$\bar{\varepsilon}$
t_{abs}, min	9,186-9,044	9,115	0,0057	0,026	0,071	0,28	0,78
V_{abs}, μl	918,6-904,4	911,5	5,67	2,53	7,04	0,28	0,77
Coefficient of peak symmetry, Ks	0,92-1,07	0,99	0,061	0,027	0,076	2,74	7,64
Coefficient of capacity ratio, k´	5,122-5,031	5,076	0,037	0,017	0,046	0,33	0,91

Table 2

The spectral ratios (S_λ / S_{210}) of atenolol (n = 5)

S_λ / S_{210}						
S_{220} / S_{210}	S_{230} / S_{210}	S_{240} / S_{210}	S_{250} / S_{210}	S_{260} / S_{210}	S_{280} / S_{210}	S_{300} / S_{210}
1,264± 0,005	1,180± 0,004	0,250± 0,004	0,048± 0,003	0,087± 0,003	0,177± 0,004	0,002± 0,004

The method of absolute calibration the peaks area of was used for the quantitative determination of atenolol by the HPLC method. To determine the range of linearity of the HPLC method of atenolol, a calibration graphs were used, which were constructed in coordinates: S, mm^2 (area of peaks) - C, μg / ml (concentration of solutions of the test substance).

The main validation characteristics of quantitative determination of atenolol - coefficients of regression of the calibration graph, correlation coefficient, interval of the linearity of the calibration graph are presented in table 3. The regression coefficients of the calibration graph equation were calculated using the least squares method. It was established that the linearity of the calibration graphs of atenolol in the coordinates (S, mm^2) - (C, μg / ml) was observed in interval of the concentrations 10,0 - 200,0 μg / ml, which corresponded to the content of preparation in the sample (4,0 μl) from 40,0 ng to 800,0 ng, respectively.

Table 3

The coefficients of regression of the calibration graphs S = вC + a of quantitative determination of atenolol by HPLC method (*P = 95%*)

The coefficients of regression of the calibration graph		Confidence intervals of coefficients of regression		Correlation coefficient (R)	The interval of the linearity of the calibration graph, μg / ml
a	**в**	**Δa**	**Δв**		
$-0,54 \cdot 10^{-2}$	$0,14 \cdot 10^{-2}$	$3,18 \cdot 10^{-3}$	$0,20 \cdot 10^{-4}$	0,9998	10,0 – 2 00,0

The given calibration graphs corresponded to the equation of the straight line, which had the forms:

$$S = 0{,}14 \cdot 10^{-2}\, C - 0{,}54 \cdot 10^{-2},$$

where S – peak area of atenolol, mm²;

C – concentration of atenolol solution, μg / ml.

It was found that the quantitative determination limit of atenolol *LOQ* is 10,0 μg / ml, which corresponds to 40,0 ng in the sample. The detection limit (*LOD*) was set at the value of the minimum analytical signal (peak height). It was established that the detection limit of atenolol by the HPLC method is equal to *LOD* = 10,0 μg /ml or 40,0 ng in sample.

As a result of determination of the content of atenolol in model solutions by HPLC method, it was established that for the developed method in investigated the concentration ranges the relative uncertainty of the average result did not exceed - $\bar{\varepsilon}$ = ± 2,21%.

For comparative evaluation of the reproducibility of analysis of atenolol by the HPLC method, studies were conducted with the change of certain conditions (analysis at different times) in the regions of low (10,0 μg / ml), medium (100,0 μg / ml) and high concentrations (200,0 μg / ml) of the studied substance: during one day of the investigation (intra-day) and during the second day (inter-day).

It was established that the values of the relative standard deviation of the results of the analysis of atenolol during (intra-day and inter-day) in the region of low concentrations was in the interval 97,2 – 102,0% ($RSD_{\bar{X}}$ did not exceed 0,75%), in the region of medium concentrations – 98,7 – 102,5 % ($RSD_{\bar{X}}$ did not exceed 0,76 %), in the region of high concentrations – 99,2 – 102,7 % ($RSD_{\bar{X}}$ did not exceed 0,77 %).

The results of quantitative HPLC determination of atenolol 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.

Conclusions

1. The identification of atenolol was conducted with using absolute parameters of retention time (t_R = 9,04 – 9,19 min), retention volume (V_R = 904,4 - 918,6 μl) and spectral ratio, which are equal: 1,264; 1,180; 0,250; 0,048; 0,087; 0,177; 0,002. The detection limit of atenolol by HPLC method was 10,0 μg / ml or 40,0 ng of sample.

2. The quantitative HPLC determination of atenolol in model solutions were conducted. It was established that the linearity of the calibration graphs of atenolol in the coordinates (S, mm²) - (C, μg / ml) was observed in interval of the concentrations 10,0 - 200,0 μg / ml, which corresponded to the content of preparations in the sample (4,0 μl) from 40,0 ng to 800,0 ng, respectively.

As a result of determination of the content of atenolol in model solutions by HPLC method, it was established that for the developed method in investigated the concentration ranges the relative uncertainty of the average result did not exceed - $\bar{\varepsilon}$ = ± 2,21%.

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