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ABSTRACT

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THE SEPARATION OF MIXTURE OF ATENOLOL, CLONIDINE, DOXAZOSIN, AMLODIPIN, ANAPRILIN BY HPLC METHOD

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Introduction. The antihyperthensive drugs such as α_1 –adrenoceptor bloking agent (doxazosin), α_2-adrenergic receptor stimulator (clonidine), β–adrenoceptor bloking agent (anaprilin), cardio-selective β_1–adrenoceptor bloking agent (atenolol), calcium-channel bloker (amlodipin) widely used both individually and in different mixtures [1].

The antihyperthensive drugs characterized not only by pharmacological action, but also by toxic effects in overdose, self-medication, can cause intoxication and fatalities. The clinical picture of poisoning and morphological changes in the body are not always characteristic and have much in common with drugs of this group [2,3]. Thus, it is important to apply the results of separation of mixtures with subsequent identification and quantification to diagnose combined poisonings of antihyperthensive drugs.

The most widely used for the analysis of mixtures of drugs is method of high performance liquid chromatography (HPLC) [4-6].

Purpose of work – the use of unified HPLC method for separation of mixture of atenolol, clonidine, doxazosin, anaprilin and amlodipin.

Materials and methods of research. Chromatographic analysis was carried out on a microcolumn liquid chromatograph "Milichrome A-02" (EkoNova, 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) 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 drugs 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 evaluation of the chromatographic separation of the mixture of the studied substances was carried out as a result of the calculation of the selectivity and the coefficient of separation of peaks on the chromatogram. The selectivity (α) was calculated according to the formula (1) given in the SPhU:

$$\alpha = k'_в / k'_а,\ k'_в > k'_а, \quad (1)$$

where $k'_а$, $k'_в$ – coefficients of capacity ratio of the analyzed substances.

Coefficient of separation of peaks (R_S) was calculated according to the formula (2):

$$R_S = \frac{1{,}18 \cdot (t_{Rb} - t_{Ra})}{b_{0.5a} + b_{0.5b}},\ t_{RB} > t_{Ra} \quad (2)$$

where t_{Ra}, $t_{Rв}$ – distances along the baseline from the injection point to the perpendiculars dropped from the peaks of two adjacent peaks, mm;
$b_{0.5\,а}$, $b_{0.5\,в}$ – width of peaks at half height, mm.

To calculate the number of theoretical plates (n) (the characteristic of the relative smearing of chromatographic zones in a column), the formula was used (3):

$$n = 5{,}54 \left(\frac{t_R}{b_{0.5}}\right)^2, \quad (3)$$

where t_R – distance along the baseline from the sample entry point to the perpendicular dropped from the maximum peak of the analyte, mm;
$b_{0.5}$ – width of peak at half height, mm.

The results of the separation of antihyperthensive drugs are presented in the chromatogram (Fig. 1).

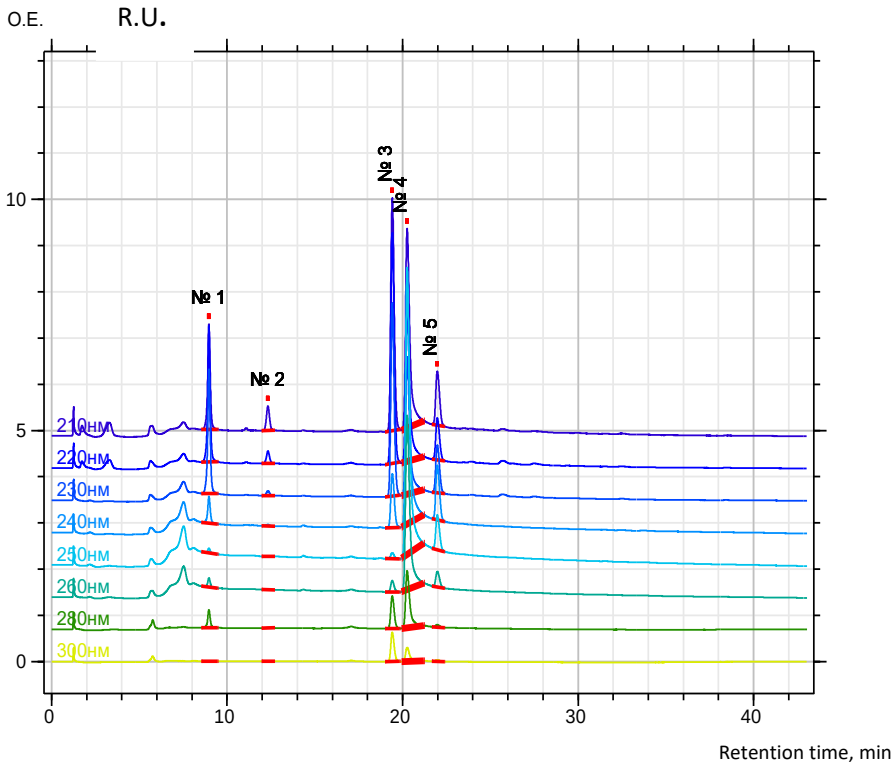

Fig. 1. Chromatographic separation of the mixture: 1 - atenolol (0,34 mg /ml); 2 – clonidine (0,03 mg /ml); 3 – anaprilin (0,038 mg /ml); 4 - doxazosin (1,14 mg /ml); 5 – amlodipin (0,38 mg /ml)

The main chromatographic parameters for the separation of the peaks of antihyperthensive drugs are given in Table 1.

Table 1

The main chromatographic parameters of the separation of the antihyperthensive drugs peaks (n = 5)

Substances	Selectivity, α				Coefficient of separation of peaks, Rs	Number of theoretical plates, *n*
Atenolol	-	-	-	-	-	1779
Clonidine	$\alpha_{2,1}$ – 1,45±0,03	-	-	-	$Rs_{2,1}$ – 3,95±0,03	3358
Anaprilin	$\alpha_{3,1}$ – 2,40±0,02	$\alpha_{3,2}$ – 1,65±0,03	-	-	$Rs_{3,2}$ – 8,37±0,03	8340
Doxazosin	$\alpha_{4,1}$ - 2,51±0,02	$\alpha_{4,2}$ – 1,73±0,03	$\alpha_{4,3}$ – 1,05±0,03	-	$Rs_{4,3}$ – 1,00±0,03	9078
Amlodipin	$\alpha_{5,1}$ - 2,74±0,03	$\alpha_{5,2}$ – 1,89±0,03	$\alpha_{5,3}$ – 1,14±0,03	$\alpha_{5,4}$ – 1,09±0,03	$Rs_{5,4}$ – 2,03±0,03	10686

The identification of antihyperthensive drugs was carried out according to the retention parameter - absolute retention time (t_{abs}): $t_{abs\ atenolol}$ = 8,96 ± 0,03 min; t_{abs}

$_{clonidine}$ = 12,31 ± 0,03 min; $t_{abs\ anaprilin}$ = 19,40 ± 0,02 min; $t_{abs\ doxazosin}$ = 20,24 ± 0,02 min; $t_{abs\ amlodipin}$ = 21,96 ± 0,03 min.

The results of evaluation of the chromatographic separation of the mixture:

selectivity was 1,05 - 2,74, coefficient of separation of peaks - 1,00 – 8,37. It has been established that for practically all substances the selectivity and the peak separation coefficients exceed 1,0.

The results of the studies indicated the suitability of unified conditions for HPLC chromatography for the separation of mixture of substances. The results of the studies can be recommended for the analysis of antihyperthensive drugs in pharmaceutical dosage forms and biological material.

Conclusions

1. The separation of mixture of atenolol, clonidine, doxazosin, anaprilin and amlodipin by unified HPLC method were conducted.

2. The results of the studies indicated the suitability of unified conditions for HPLC chromatography for the separation of mixture of substances.

3. The results of the studies can be recommended for the analysis of antihyperthensive drugs in pharmaceutical dosage forms and biological material.

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