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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF CLONIDINE BY HPLC METHOD

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The aim *of this work is identification and quantification of clonidine hydrochloride by a unified HPLC method, which allows to obtain reliable and reproducible research results.*

Materials and methods. *HPLC analysis was carried out on a microcolumn liquid chromatograph "Milichrome A-02" in conditions: reversed-phase variant, column with non-polar sorbent Prontosil 120-5 C_{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. The flow rate of the mobile phase has been formed 100 μl/min, injection volume – 4 μl. Multichannel detection of the substance was carried out using a UV detector at 210, 220, 230, 240, 250, 260, 280 and 300 nm; the optimal value of column temperature – 37 - 40 ℃ and pressure of pump – 2.8 – 3.2 MPa.*

Results and its discussion. *As a result of studies using a unified HPLC method, were obtained the retention parameters of clonidine hydrochloride and spectral relationships, which made it possible to include the results obtained in the database for the identification of antihypertensive drugs in the therapeutic monitoring of treatment with an individual drug, or comprehensive treatment of diseases of the cardiovascular system. The development of the quantitative determination of clonidine hydrochloride by HPLC on model solutions using various concentrations of the drug was carried out. The content of clonidine hydrochloride was determined according to the equation $S = 0.5 \cdot 10\text{-}4\ C + 1.8 \cdot 10\text{-}3$; the correlation coefficient was 0.9964. It is established that the relative uncertainty of the average result did not exceed $\pm$2.12 % when HPLC analysing of clonidine hydrochloride in model solutions.*

Conclusions. *Identification and quantification of clonidine hydrochloride by a unified HPLC method, which allows to obtain reliable and reproducible research results were conducted. The results of research by a unified HPLC method can be recommended for implementation in the practice of forensic bureaus, toxicological centers, clinical laboratories for the study of drugs in biological objects*

Keywords: *clonidine hydrochloride, analysis by a unified HPLC method*

1. Introduction

Clonidine hydrochloride – N–(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine hydrochloride is the antihypertensive drug. Clonidine is used to treat hypertensive crisis, arterial hypertension. The hypotensive effect of clonidine is accompanied by a decrease in cardiac output and a decrease in the peripheral resistance of blood vessels, including blood vessels of the kidneys. Clonidine also causes a decrease in intraocular pressure, associated with a decrease in secretion and improved outflow of aqueous humor [1, 2].

Due to its effect on the central nervous system, clonidine has an analgesic and sedative effects. Wang J. G. and co-authors were conducted studies that confirmed the efficacy and safety of clonidine as a sedative in critically ill patients with artificial lung ventilation [3]. Hayden J. C. and co-authors were proved the effectiveness of the use of clonidine as a sedative in children with artificial lung ventilation [4].

Clonidine is used to treat withdrawal symptoms in opioid addiction. Kowalczyk W. J. and co-authors were obtained positive results with daily clonidine intake as an adjunct to buprenorphine therapy for opioid dependence [5].

In case of overdose and self-medication, clonidine can cause severe intoxication, which is accompanied by suppression of the functions of the central nervous system, cardiovascular and respiratory systems with the development of hypotension, bradycardia, collapse, coma. Najami O. L. and co-authors found that a pronounced toxic effect occurred in children as a result of a single dose of 0.4–4 mg, and in adults – 4.5–11.25 mg of clonidine [6].

Clonidine is known in criminal practice as a drug that criminals use to add to alcohol and euthanize the victim for robbery and murder [6].

Isbister G.K. and co-authors conducted a retrospective review of the clinical database or medical records of poisoned patients who suffered from clonidine overdose (> 200 μg). Clinical effects, treatment, complications of clonidine poisoning have been established [7].

Clonidine is becoming increasingly popular for the treatment of behavioral disorders in children. Clonidine has a narrow therapeutic index and toxicity can occur with accidental double ingestion. According to the data of Cairns, R. with co-authors, when dosing the drug, increased vigilance is required, which is due

to the high incidence of children under the action of clonidine [8].

The use of combined drugs with clonidine is dangerous for the health of children. Schmitt C. with co-authors was studied a case of toxic damage to a 9-year-old boy during a growth hormone test: he received a tenfold dose of clonidine (0.23 mg instead of 0.023 mg) in combination with 6.2 mg of lbetaxolol [9]. Cates A. L. with co-authors was described a case of toxic damage of a 22-month-old girl who drank a small amount of a therapeutic cream containing camphor, gabapentin, clonidine, ketoprofen and lidocaine [10].

Considering the widespread use of clonidine in medical practice and its toxic effect, the development of effective and economical methods of analysis is an actual task. Among modern methods for the analysis of drugs, one of the most highly sensitive and selective is the method of high performance liquid chromatography (HPLC), which is widely used to diagnose drug intoxication and monitor the effectiveness of treatment of the population.

The literature contains HPLC methods for the study of clonidine under various conditions (options for detecting the test substance, the use of isocratic and gradient elution modes, the use of different compositions of mobile phases, sorbents, buffer solutions), which are based on the individual properties of the drug, but they do not take into account the possibility of treatment with mixtures of different drugs and combined intoxications.

Egorova A.V. and co-authors developed an HPLC method for the determination of traces of clonidine in washes during the cleaning of pharmaceutical equipment [11]. According to this technique, chromatography was carried out chromatograph Agilent 1200 3D LC System C with diode array detector in a reversed-phase version using 4.6 mm x 250.0 mm steel column filled with a 5 μm Zorbax Eclipse XDB-C18 sorbent. Elution was carried out in an isocratic mode using a mobile phase – 0.22 % solution of sodium octane sulfonate – methanol – acid phosphoric (400:600:1.0). The analysis of substances was carried out only at one wavelength – 220 nm. The retention time of clonidine was 2.8 min.

According to the State Pharmacopoeia of Ukraine, HPLC analysis of the drug substance clonidine hydrochloride was carried out using 3.0 mm x 150.0 mm steel column filled with a 5 μm sorbent – propylsilyl silica gel [12]. Elution was carried out in an gradient mode using a mobile phase – A – phosphate buffer solution pH 4.0 and B – phosphate buffer solution – acetonitrile (25:75). Elution programme: from the beginning of the process (90:10) to 15 min (30:70), from 15 to 15.1 min to (90:10), and from 15.1 to 20 from to initial conditions. Flow rate: 1.5 ml/min. The analysis of substances with a UV spectrophotometric detector was carried out only at one wavelength – 210 nm.

Tang F. and co-authors was developed an HPLC method in combination with tandem mass spectrometry for the simultaneous quantitative assessment of clonidine, morphine and its main metabolites for pharmacokinetic analysis in a clinical study comparing the use of morphine and clonidine in newborns diagnosed with withdrawal symptoms [13]. The preparations were separated on a column Inertsil ODS-3 (4 μm) using 0.1 % formic acid in water and 0.1 % formic acid in a methanol gradient.

De Nicolò A. and co-authors was developed an HPLC method for the analysis of antihypertensive drugs in urine: amlodipine, atenolol, clonidine, chlorthalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril and telmisartan for therapeutic monitoring [14]. Chromatographic separation was performed on a column Acquity® UPLC HSS T3 1.8 μm 2.1 mm × 150 mm with a gradient of water and acetonitrile, to which 0.05 % formic acid was added.

For the analysis of clonidine in biological objects Clarke, E. J. C. different chromatographic conditions were recommended [2]. The investigation was carried out in a reversed-phase variant using a steel column 4.6 mm x 250.0 mm filled with an C_8 Symmetry, 5 μm sorbent with Symmetry C_{18} pre-column (20 mm). Elution was carried out in a gradient mode using eluent A (phosphate buffer (pH 3.8) and eluent B (acetonitrile). Elution programme: from (85:15) to (65:35) for 6.5 min, from (65:35) to (20:80) for 25 min, (20:80) hold for 3 min and back to initial conditions for equilibration for 7 min. Flow rate: 1 ml/min for 6.5 min, then linear increase to 1.5 ml/min for 6.5 to 25 min and hold for 3 min. The analysis of substances was carried out only at one wavelength – 229 nm. The retention time of clonidine was 6.1 min.

For the analysis of clonidine in the isocratic mode, the following procedure was recommended: chromatography was carried out in a reversed-phase variant using a steel column 4.6 mm x 250.0 mm filled with an Supelcosil LC-DP, 5 μm sorbent. Elution was performed in an isocratic mode (25:10:5) using eluent A (acetonitrile), eluent B (phosphoric acid (0.025 % v/v) and eluent C (triethylamine buffer). Flow rate: 0.6 ml/min. The analysis of substances was carried out only at one wavelength - 229 nm. The retention time of clonidine was 7.8 min.

Modern HPLC methods of clonidine analysis indicate the absence of systematic studies, which does not allow the selection of optimal conditions for drug analysis in biological objects and pharmaceuticals.

The aim of this work is identification and quantification of clonidine hydrochloride by a unified HPLC method, which allows to obtain reliable and reproducible research results.

2. Planning (methodology) of research

The previously presented methods of HPLC analysis of clonidine have disadvantages. The use of an isocratic elution mode limits the possibility of all sample components leaving the column in narrow zones and efficient separation of drug mixtures [2, 11]. The use of a nonlinear gradient in the elution of clonidine complicates the chromatography process [2, 12].

Detection of a drug at one wavelength reduces the reliability of the results obtained during identification, since it allows using only retention parameters without taking into account spectral ratios [2, 11, 12]. HPLC methods for the analysis of clonidine have limitations in their application to the study of mixtures with other drugs.

Given the possibility of comprehensive treatment of diseases of the cardiovascular system with the

use of various drugs for the analysis of clonidine by a unified HPLC method is an urgent task of the study.

The presented study included the following stages: establishment of clonidine hydrochloride retention parameters, spectral ratios and limits of drug detection in the sample; elaboration of HPLC-method of quantitative determination of clonidine hydrochloride on model solutions using different concentrations of the drug; calculation of validation characteristics of HPLC method for determination of clonidine hydrochloride: range of linearity, limits of quantitative determination, accuracy and precision based on the results of quantitative determination of the drug by HPLC method in model solutions.

3. Materials and methods

Solvents for HPLC study met the qualification "for HPLC": acetonitrile (Sigma-Aldrich Laborchemikallen, GmbH), methanol (Merk, Germany), water is double-distilled (Merk, Germany). Reagents corresponded to the qualification of "PFA": lithium perchlorate trihydrate (Sigma-Aldrich, USA), (70 %) acid perchloric (Chimmed, Russia).

The standard methanol solution of clonidine hydrochloride with concentration of 100.0 μg / ml was used in the study on the basis of the Kharkiv Regional Bureau of Forensic Medicine. Chromatographic analysis was carried out on a microcolumn liquid chromatograph "Milichrome A-02" (EkoNova, Closed Joint-Stock Company, Russia) according to the unified HPLC methodology developed by the author – Baram G.Y. [15, 16].

The reversed-phase variant of chromatography is used as an option with a high speed of the establishment of sorption equilibrium, the ease and completeness of the desorption of components from a non-polar sorbent in small volumes of solvent. The studies were carried out on a 2 mm × 75 mm column with a non-polar sorbent Prontosil 120-5 C_{18} AQ, 5 μm. Prontosil sorbent has a high sorption capacity, inertness to substances, mechanical strength, heat resistance.

Mobile phase included organic solvent and a buffer solution. Acetonitrile has been filtered through the MPA-MA-N-2 (TU 6-05-1909-81) membrane with a pore size of 0.15–0.25 μm, degassed by use of vacuum. Buffer solution included ion-pair agent – 0.2 M solution of lithium perchlorate in 0.005 M solution of acid perchloric, which before using was diluted in 25 times with the potentiometric setting of value pH 3.0 by adding 0.005 M solution of acid perchloric.

Gradient elution with mixtures of solvents was a linear gradient from eluent A (5 % acetonitrile and 95 % buffer) to eluent B (100 % acetonitrile) for 40 min. The gradient mode provided a decrease in the eluent polarity with the addition of a less polar solvent (acetonitrile) and reduced components retention. The gradient mode created the conditions for the exit from the column of all components of the sample in the form of narrow zones. The final stage of the gradient corresponded to a phase with high content of acetonitrile. Regeneration of the column was performed for 2 min with a mixture of solvents (2 % acetonitrile and 98 % buffer solution).

The thermostat of column of solid-state type with an electric heater provided optimum conditions of chromatography and reproducibility of the results. The optimum pressure of pump is 2.8–3.2 MPa; the flow rate of the mobile phase was 100 μl/min; optimum temperature value – 37–40 °C. The volume of the samples for injection was 4 μl.

The detection of a substance after it leaves the column was carried out with the use of a dual-beam multi-wave UV spectrophotometer in the wavelength range 190–360 nm, the accuracy of the wavelength of 0.5 nm. For multichannel detection of substances are recommended wavelengths: 210, 220, 230, 240, 250, 260, 280 and 300 nm. For each value of the wavelength on the chromatogram of the substances, an appropriate peak with the same retention time was observed, but with different amplitudes, directly proportional to the extinction of a substance (the absorption coefficient of electromagnetic radiation at a given wavelength).

Symmetrical, acute peaks on chromatograms were obtained by applying a unified HPLC technique for the analysis of clonidine hydrochloride, which allowed to calculate the results using the computer program "Multi-Chrom" ("Ampersend", Russia), which was part of the chromatograph (Fig. 1).

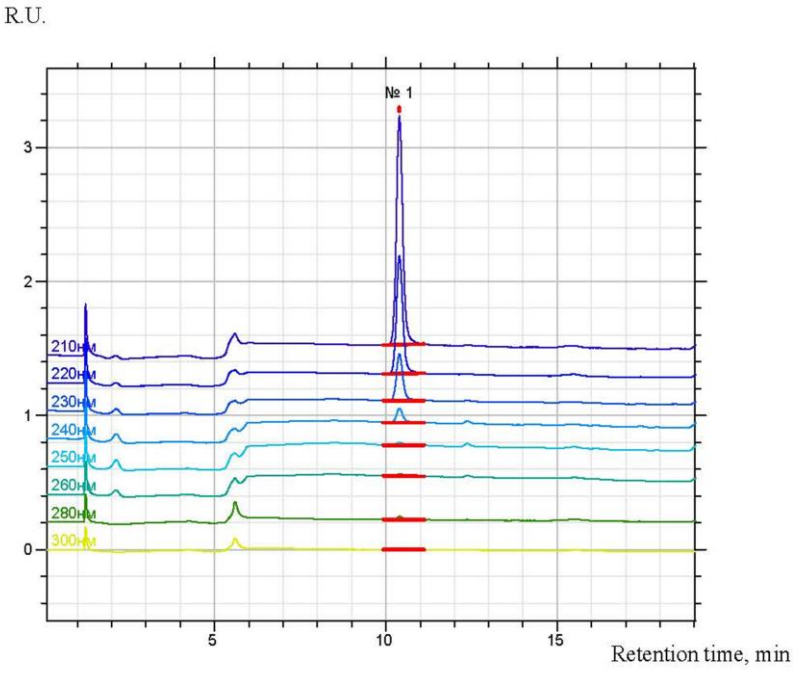

Fig. 1. Chromatogram of clonidine hydrochloride (concentration – 100.0 μg/ml)

4. Results

The identification of clonidine hydrochloride conducted with using absolute parameters of retention time (t_R) and retention volume (V_R) (Table 1).

The suitability of the chromatographic system for HPLC studies of clonidine hydrochloride was confirmed in determining the coefficients of the symmetry of the peaks of the substance (*Ks*) (not exceeding the optimal values of 2.0–2.5); coefficients of capacity ratio (*k'*) (were not less than values of 0.5–2.0).

The spectral ratio values absorbance at wavelengths (S_λ/S_{210}) – from 220 to 300 nm – the values of absorbance at 210 nm are presented in the Table 2.

Table 1

Retention parameters of clonidine hydrochloride obtained as the result of HPLC analysis (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	10.06–11.00	10.53	0.41	0.17	0.47	1,74	4.51
V_{abs}, μl	1005.82–1100.78	1053.3	41.1	17.08	47.48	1,74	4.51
Coefficient of peak symmetry, Ks	0.89–0.99	0.94	0.037	0.017	0.047	1.76	5.02
Coefficient of capacity ratio, k´	5.70–6.34	6.02	0.26	0.12	0.32	1.93	5.29

Table 2

The spectral ratios (S_λ/S_{210}) of clonidine hydrochloride (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}
Clonidine hydro chloride	0.515±0.005	0.207±0.004	0.064±0.005	0.017±0.003	0.014±0.003	0.016±0.004	0.014±0.003

Comparative assessment of spectral ratios makes it possible to obtain more reliable and reproducible results, as well as to identify drugs with similar values of retention parameters.

The HPLC method of determination of clonidine hydrochloride is validated by parameters: linearity range, detection limit (*LOD*), quantification limit (*LOQ*), accuracy and precision in the regions of low, medium and high concentrations of the substances [17]. The method of absolute calibration the peaks area of clonidine hydrochloride was used for the quantitative determination by the HPLC method.

To determine the range of linearity of the HPLC method of clonidine hydrochloride, a calibration graph was constructed in coordinates: S, mm^2 (area of peaks) - C, μg/ml (concentration of solutions) (Fig. 2).

For HPLC-analysis of clonidine hydrochloride in a series of volumetric flasks of capacity 50.0 ml were introduced from the burette of 2.5; 5.0; 10.0; 15.0; 25.0; and 37.5 ml of standard solution and the volumes of solutions were brought to the mark with the appropriate solvent (working standard solutions 1–7 had concentrations of 5.0; 10.0; 20.0; 30.0; 50.0; 75.0 and 100.0 μg/ml, respectively).

HPLC studies were performed with the application of the proposed method, the volume of the sample was 4.0 μl. Five parallel HPLC measurements were performed for each concentration of preparation in solutions.

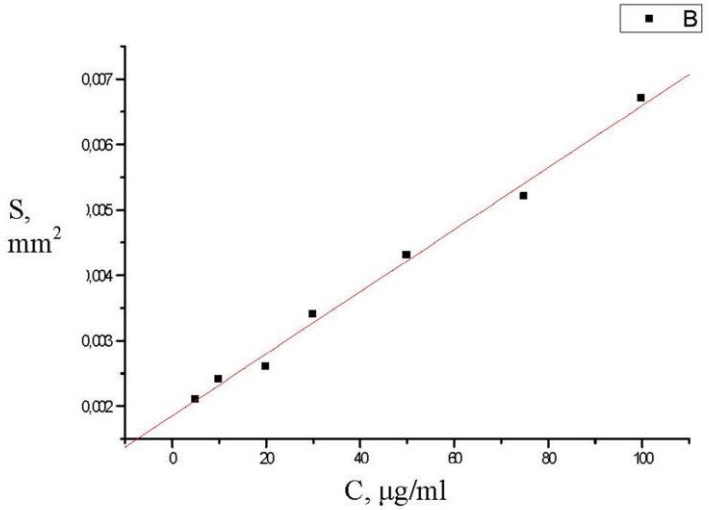

Fig. 2. Calibration graph of quantitative determination of clonidine hydrochloride by HPLC method

The main validation characteristics of quantitative determination of clonidine hydrochloride – 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.

Table 3

The coefficients of regression of the calibration graph S=cC+a of quantitative determination of clonidine hydrochloride by HPLC method (*P=95 %, n=7*)

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	c	Δa	Δc		
$1.85 \cdot 10^{-3}$	$0.5 \cdot 10^{-4}$	$0.10 \cdot 10^{-3}$	$0.18 \cdot 10^{-7}$	0.9964	5.0–100.0

It was established that the linearity of the calibration graph of clonidine hydrochloride in the coordinates (S, mm^2) – (C, μg/ml) was observed in interval of the concentrations 5.0–100.0 μg/ml, which corresponded to the content of preparations in the sample (4.0 μl) from 20.0 ng to 400.0 ng, respectively. It was established that the quantification limit of clonidine hydrochloride by the HPLC method is equal to 5.0 μg/ml or 20.0 ng in sample.

The equation of the line $S=0.5 \cdot 10^{-4}\ C+1.8 \cdot 10^{-3}$ corresponds to the indicated calibration graph. The correlation coefficient (R) was equal to 0.9964.

The detection limit was set at the value of the minimum analytical signal (peak height). The signal exceeding 2 times the fluctuation noise level was used as the minimum peak height. The detection limit was set when applying the signal / noise ratio (S/N), which was equal to the ratio of the double peak height (2H) to the background noise region on the chromatogram of the blank solution. It was established that the detection limit of clonidine hydrochloride by the HPLC method is equal to 5.0 μg/ml or 20.0 ng in sample.

Results of quantitative determination of clonidine hydrochloride in model solutions by HPLC method are presented in Table 4.

The relative uncertainty of the average result did not exceed ±2.12 % when HPLC analysing of clonidine hydrochloride in model solutions using the proposed method.

The accuracy and precision of the HPLC – technique of quantitative determination of clonidine hydrochloride were established by the values of the relative standard deviation of the average result in percentages – $RSD\bar{x}$ 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 clonidine hydrochloride in the interval of linearity of the calibration graph in model solutions did not exceed 1.0, which indicated the proximity of the results of the analysis to their true value.

The studies were conducted on samples of a single series of drugs by one analyst under identical conditions (reagents, equipment, laboratory) over a short period of time, which confirmed the convergence of the results.

For comparative evaluation of the reproducibility of analysis of clonidine hydrochloride 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 (50.0 μg/ml) and high concentrations (100.0 μg/ml) of the studied substance: during one day of the investigation (intra-day) and during the second day (inter-day). Results of accuracy and precision analysis of quantitative determination of clonidine hydrochloride in model solutions by HPLC method are presented in Table 5.

Table 4

Results of quantitative determination of clonidine hydrochloride in model solutions by HPLC method (n=5, *P*=95 %)

Concentration of the solution, μg / ml	S, mm^2	Amount of substance determined,		Metrological characteristics
		μg	%	
5.0	0.00207	4.92	98.4	$\bar{X} = 99.86$, $S^2=2.89$
20.0	0,00263	20.24	101.2	S=1.70 $S\bar{x} = 0.76$
50.0	0.00430	50.0	100.0	$RSD\bar{x} = 0.74$
75.0	0.00529	76.35	101.8	$\Delta\bar{x} = 2.11$
100.0	0.00656	97.90	97.9	$\bar{\varepsilon} = 2.12$ $\bar{X} \pm \Delta\bar{x}$ =99.86±2.11 %

Table 5

Accuracy and precision of the quantitative determination of clonidine hydrochloride in model solutions by HPLC method (n=5, *P*=95 %)

Concentration of the solution, μg/ml	Amount of substance determined, %	Metrological characteristics					
		$\bar{X}$	*S*	$S\bar{x}$	$\Delta\bar{x}$	$RSD\bar{x}$	$\bar{\varepsilon}$
Intra-day							
10.0	97.3–101.1	99.2	1.54	0.69	1.92	0.69	1.93
50.0	98.8–103.0	100.9	1.70	0.76	2.12	0.75	2.10
100.0	98.8–102.8	100.8	1.64	0.73	2.03	0.73	2.02
Inter-day							
10.0	98.1–102.3	100.2	1.70	0.77	2.11	0.76	2.11
50.0	97.9–102.3	100.1	1.77	0.79	2.20	0.79	2.20
100.0	97.3–101.9	99.6	1.86	0.83	2.31	0.83	2.32

It was established that the values of the relative standard deviation of the results of the analysis of clonidine hydrochloride during one day (intra-day) in the region of low concentrations was in the interval 97.3–101.1 % ($RSD_{\bar{x}}$ did not exceed 0.69 %), in the region of medium concentrations – 98.8–103.0 % ($RSD_{\bar{x}}$ did not exceed 0.75 %), in the region of high concentrations – 98.8–102.8 % ($RSD_{\bar{x}}$ did not exceed 0.73 %).

It was established that the values of the relative standard deviation of the results of the analysis of clonidine hydrochloride during second day (inter-day) in the region of low concentrations was in the interval 98.1–102.3 % ($RSD_{\bar{x}}$ did not exceed 0.76 %), in the region of medium concentrations – 97.9–102.3 % ($RSD_{\bar{x}}$ did not exceed 0.79 %), in the region of high concentrations – 97.3–101.9 % ($RSD_{\bar{x}}$ did not exceed 0.83 %).

The precision of the quantitative determination of clonidine hydrochloride in model solutions by HPLC method was studied as a result of research intra-day and inter-day. It was established, that the relative standard deviation $RSD_{\bar{x}}$ did not exceed 0.83 %, which indicated the accuracy of the analysis.

5. Discussion

As a result of studies using a unified HPLC method, were obtained the retention parameters of clonidine hydrochloride (t_{abs}=10.06–11.00 min; V_{abs}=1005.82–1100.78 μl) and spectral relationships (0.515, 0.207, 0.064, 0.017, 0.014, 0.016, 0.014), which made it possible to include the results obtained in the database for the identification of antihypertensive drugs in the therapeutic monitoring of treatment with an individual drug, or comprehensive treatment of diseases of the cardiovascular system.

The development of the quantitative determination of clonidine hydrochloride by HPLC on model solutions using various concentrations of the drug was carried out. The content of clonidine hydrochloride was determined according to the equation

$$S = 0.5 \cdot 10^{-4} C + 1.8 \cdot 10^{-3};$$

the correlation coefficient was 0.9964.

It is established that the relative uncertainty of the average result did not exceed $\pm$2.12 % when HPLC analysing of clonidine hydrochloride in model solutions.

Calculation of the validation characteristics of the HPLC method for the determination of clonidine hydrochloride made it possible to establish the range of linearity (5.0–100.0 μg/ml), the limit of quantification (5.0 μg/ml or 20.0 ng in sample), correctness and accuracy of research (the relative standard deviation $RSD_{\bar{x}}$ did not exceed 0.83 %, which indicated the accuracy of the analysis).

The disadvantage of this methodology are the weak material base of modern medical institutions, which limits its practical implementation. Changing the conditions of the analysis using a unified methodology will violate the reliability and reproducibility of the results.

Study limitations. The limitation in the use of the HPLC method for the analysis of clonidine in biological objects is the lack of an elaborated preliminary purification of extracts from biogenic impurities, since accompanying substances in the biological material can significantly affect the results of the study.

The limitation of the use of the developed technique is also the lack of equipment and reagents for HPLC research in appropriate medical institutions for its practical implementation.

Prospects for further research. The elaborated HPLC method for the analysis of clonidine can be recommended for implementation in the practice of the Bureau of Forensic Medicine when conducting research on drugs found near the corpse.

At the same time, the developed technique is part of the algorithm for studying clonidine in biological objects, which includes isolation, purification, identification, and quantitative determination of the substance. After conducting experimental studies in biological material on the use of the HPLC method for the analysis of clonidine, the obtained data can be recommended for implementation in the practice of toxicological centers, clinical laboratories for the study of drugs in biological objects.

6. Conclusions

1. Identification and quantification of clonidine hydrochloride by a unified HPLC method, which allows to obtain reliable and reproducible research results were conducted.

2. The validation characteristics of the HPLC method for the determination of clonidine hydrochloride are calculated: the range of linearity, the limits of detection and quantification, the accuracy and precision of the results of quantitative determination of the drug by HPLC in model solutions.

3. The results of research by a unified HPLC method can be recommended for implementation in the practice of forensic bureaus, toxicological centers, clinical laboratories for the study of drugs in biological objects.

Conflict of interests

The authors declare that they have no conflicts of interest.

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