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IR–SPECTROSCOPIC METHOD TO HELP IN CHEMICAL–TOXICOLOGICAL INVESTIGATION OF AMLODIPINE

Abstract. Diseases of the cardiovascular system are very common among the population today. According to statistics, 17 million people live in the world every year. One of the main reasons for this is acute heart rhythm disturbances, problems with high blood pressure. In particular, ventricular tachycardia and fibrillation of cardiac arrhythmias are very life–threatening. Therefore, in connection with life –threatening severe rhythm changes, such as tachycardia and irreversible ventricular fibrillation, the development of suitable antiarrhythmic drugs that can be fast and highly effective even in severe arrhythmias remains one of the urgent tasks of today. Based on this, the relevance of our work is the development of methods for separating amlodipine from a biological object, improvement of methods for analyzing the drug amlodipine, enalapril and metaprolol which is widely used in the treatment of hypertensive diseases.

Keywords: hypertension, biological object, substance, tablet, drug, blood, urine, methods of analysis, solvent system.

Actuality of theme. Diseases of the cardiovascular system are very common among the population today. According to statistics, 17 million people live in the world every year (Oshchepkova E. V., 2007). One of the main reasons for this is acute heart rhythm disturbances, problems with high blood pressure. In particular, ventricular tachycardia and fibrillation of cardiac arrhythmias are very life –threatening (Wathen M. S., 2004; Anthony R., 2008; Knecht S., 2009; Juan J. S.M., 2010). Therefore, in connection with life–threatening severe rhythm changes, such as tachycardia and irreversible ventricular fibrillation, the development of suitable antiarrhythmic drugs that can be fast and highly effective even in severe arrhythmias remains one of the urgent tasks of today. Based on this, the relevance of our work is the development of methods for separating amlodipine from a biological object, improvement of methods for analyzing the drug amlodipine, enalapril and metaprolol which is widely used in the treatment of hypertensive diseases.

Communication of dissertation work with research plans. Practise an assay method that separates the drug from the biological object, causing hypersensitivity reactions to the drug amlodipine, which lowers blood pressure and normalizes heart function in hypertensive patients.

The purpose of the study.

1. Development of hypersensitive chemical reactions to antihypertensive drugs;

2. To determine the sensitivity of the reaction to antihypertensive drugs;

3. Improvement of methods of quantitative analysis of antihypertensive drugs;

4. Development of a method for separating antihypertensive drugs from biological fluids and biological objects.

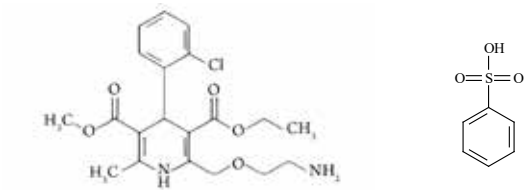
Object and subject of research. Antihypertensive drugs that lower blood pressure.

Research methods: during the analysis, the use of chemical reactions, the creation of qualitative reactions to the preparations obtained for the analysis, the use of thin layer chromatography, spectrophotometric and high–efficiency liquid chromatography methods, as well as the use of infrared spectroscopic spectroscopy methods.

Scientific, theoretical and practical significance of the research results. Implementation of the developed methods of analysis in the Republican laboratories of forensic chemistry.

Introduction

Amlodipine is a derivative of digiropyridine and has antianginal and hypotensive effects. At the same time, it causes a number of unpleasant reactions, and in some cases cases of poisoning have also been observed. As a result of excessive vasodilatation, there is a sharp drop in arterial pressure and tachycardia.



$C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$ mm=567.1

(RS)–2–(2–aminoethoxymethyl)–6–methyl 4–(2–chlorophenyl)–1,4–dihydropyridine – benzenesulfonate of 3,5–dicarboxylic acid.

Amlodipine is widely used in medical practice as a hypotensive agent. In some cases, acute poisoning occurs when it is taken in excess of the norm. In such cases, the patient's urine and blood are analyzed and a conclusion is drawn. Determination of the authenticity of the substance was carried out by the method of thin layer chromatography. When amlodipine is isolated from biological liquid urine and determined by TLC method, 2 g of ammonium sulfate is added to 15 ml of urine containing 10 mg of the substance, extracted 3 times (5 ml) with chloroform, 1 g of anhydrous sulfate is filtered through a filter containing sodium sulfate, and washed with 2 ml of chloroform. The chloroform was evaporated to a dry residue. The residue was dissolved in 2 ml of alcohol, then the solution was dripped onto the starting line of a silufol UV-254 plate with a diameter of 3 mm using a capillary and dried. Then 1 mol was chromatographed in a chromatographic chamber saturated with acetic acid vapor. When the plate was dried from the chamber and exposed to iodine vapor, a blue spot with Rf=0.87 was observed. At the same time, an IR spectroscopic method for determining the authenticity and quantity of the substance extracted from the biological fluid by the method described above was developed.

Basic phrases. Amlodipine, thin layer chromatography, silufol – UV-254, IR –spectroscopy, valence and deformation vibrations, intensity of absorption path, validation.

The purpose of the study. Chemical–toxicological studies of amlodipine are of great importance, allowing quick and accurate investigation of cases of poisoning. We aimed to develop an infrared spectroscopic method for the extraction and analysis of amlodipine from biological fluids.

Practical part. From the IR spectrum of amlodipine isolated from biological fluid, the following classification absorption pathways were determined: n max: cm –1, 3301 primary NH_2 group, 3190 (pyridine ring N–H valence vibrations amino group valence vibrations), 2981 (C–H valence vibrations in ethyl and methyl groups); 2948 (valence vibrations of C–H bonds in aromatic ring and pyridine ring) 1698, 1675 (valence vibrations of carbonyl bonds in carboethoxyl group, 1615 (valence vibrations of aromatic ring – C=C bonds), 1493 (valence vibrations of C=C bonds in the pyridine ring), 1445, 1432 (deformation vibrations of methyl groups), 1384, 1365 (deformation vibrations of C–H groups in the aromatic ring and pyridine ring), 1303, 1265 (out–of–surface defor-

mation vibrations of C–H groups), 1202, 1125 (plane deformation vibrations of C=C bonds to aromatics), 1095, 1034, 1049 (carboxyl and oxyethiamine and categories C–O–), (1,2, Plane strain vibrations of 4,5–substituted pyridine C=C bonds, 869, 838, 791 (C–H bonds of 1, 2, 3 substituted aromatic rings), 735, 615 cm-1 (C–H valence vibrations).

Quantitative analysis. Infrared spectra of a 1 mg (exact draw) amlodipine working standard sample and samples isolated from a biological object were obtained in the form of pressed KBr tablets, and the areas of carboethoxyl groups were measured as characteristic absorption pathways.

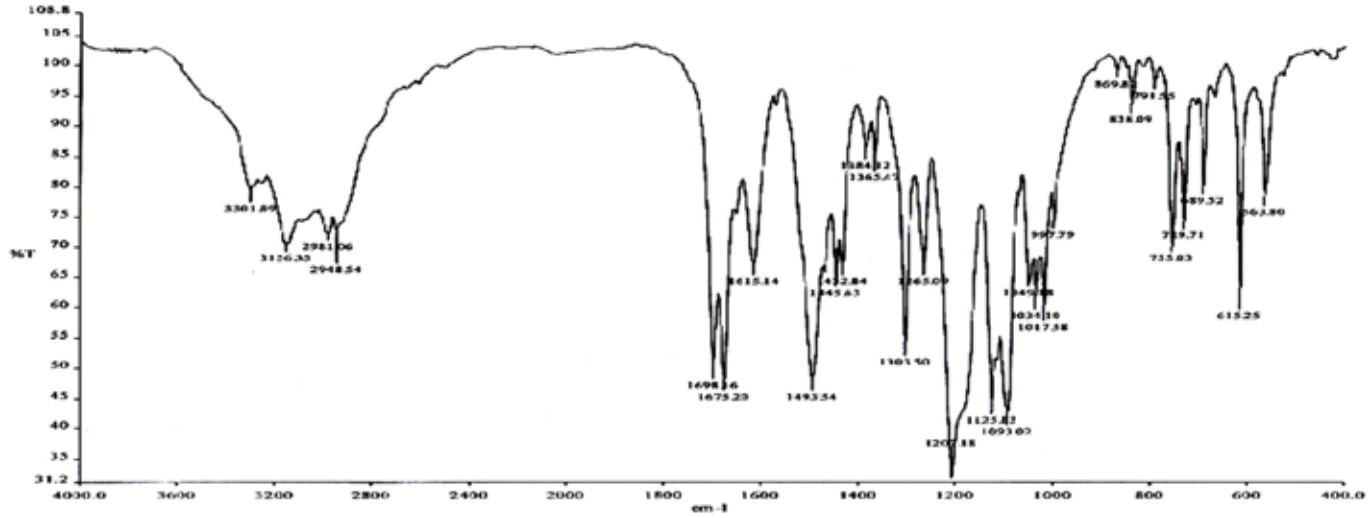


Figure 1. – IR spectrum of amlodipine isolated from biological fluid

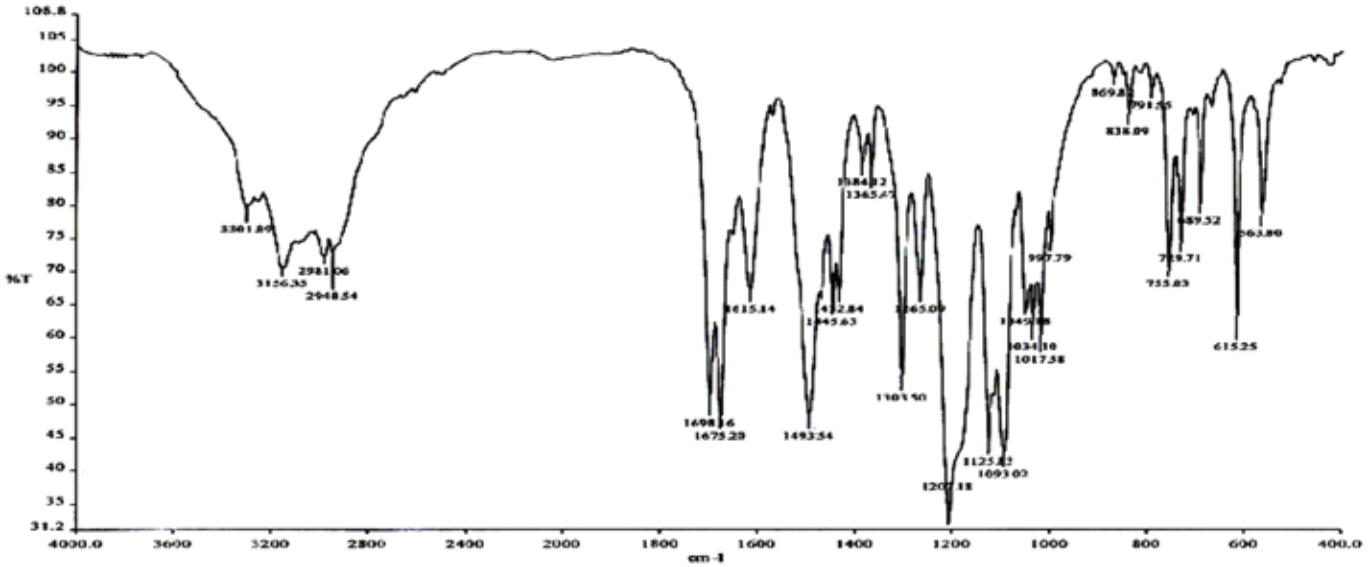


Figure 2. IR–spectrum of a working standard sample of amlodipine

The percentage of amlodipine extracted from the biological object was determined using the following calculation formula.

$$X = \frac{S \cdot m_0 \cdot P \cdot 100}{S_0 \cdot m \cdot 100}.$$

in the formula: S – the area of absorption paths of carbonyl groups in the IR spectrum of the standard sample;

S_0 – amlodipine working standard sample IR – area of absorption paths of carbonyl groups in the spectrum;

m – Drawer of amlodipine isolated from a biological object;

m_0 – drawer of the woing standard sample of amlodipine;

P – amlodipine is the percentage of amlodipine in the working standard sample.

Metrological description and validation of the analytical method

Table 1.

№	*m*	m_0	*X*	$\bar{X}$	S^2	*S*	Δ*x*	Δ $\bar{X}$	*E*%	$\bar{E}$ %	*RSD*%	*V*%
1	0.00090		98.4									
2	0.00095		98.7									
3	0.00098	0.00010	99.2	99.2	0.32	0.57	1.57	0.7	1.71	0.76	0.6	0.32
4	0.00103		99.6									
5	0.00107		100.2									

Specificity of the method. When the spectra obtained at different concentrations and with different equipment are compared, the intensity and classification values of the absorption lines in them are the same, and the absorption lines of the carbonyl groups in the spectra were observed at 1698, 1675 cm^{-1}.

Linearity of the method. The linear relationship was determined in the dimensions of the relationship between the draw and the characteristic absorption path for obtaining an IR–spectrum, the correlation coefficient was 0.9992 nm, and the graph of the linear relationship was found to be $y = 3.5833 \times \times - 0.0167$.

IR–spectrometric quantitative analysis method of amlodipine linearity of the relationship between the amount of substance and the area of the absorption path

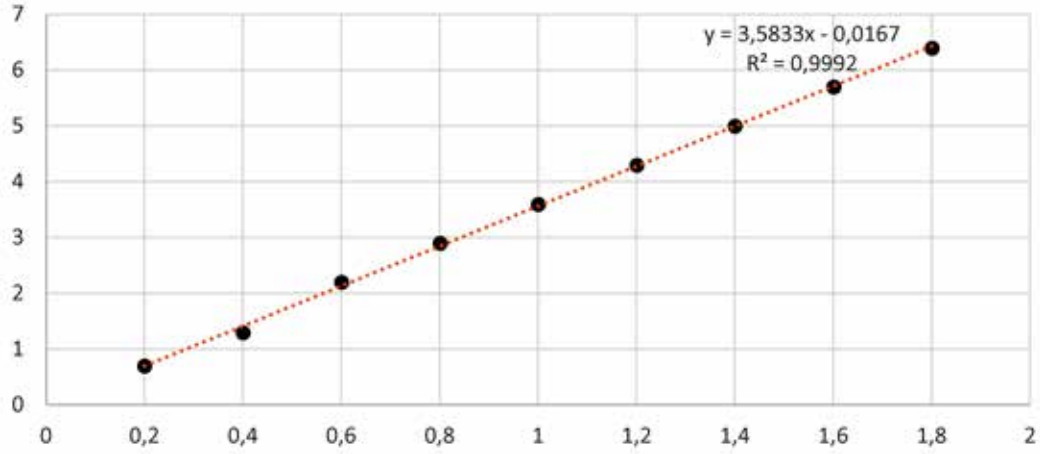


Figure 3.

The precision of the method was determined in six re–analyses and was determined by studying the metrological description of the results.

Table 3.

X_g	*X*%	$\bar{X} - X_i$	d_2	**Metrological description**	
1	***2***	***3***	***4***	***5***	
0.00090	98.4	1.2	1.44	$S^2 = 0.75$	$\Delta X = 0.69$
0.00095	98.7	0.5	0.25	$S = 0.27$	$\Delta \bar{X}$=0.28

1	*2*	*3*	*4*	*5*	
0.00098	99.2	0	0	$S_{\bar{X}} = 0.11$	$E = 0.27$
0.00104	99.6	0.4	0.16	$\bar{E}$=0.12	V=0.75
0.00108	100.2	1.0	1.0	RSD=0.8%	

The reproducibility of the method was carried out by analyzing the samples extracted from the biological fluid six times on the basis of triplicate experiments, and processing the obtained values with the method of mathematical statistics.

Table 4.

Return № number	**Percentage of Amlodipine**		
	Experiment 1	**Experiment 2**	**Experiment 3**
1	98.4	98.1	98.2
2	98.7	98.8	98.6
3	99.2	99.4	99.8
4	99.6	99.7	100.2
5	100.2	100.5	100.8
6	100.5	101.4	101.8
RSD	0.8	2.1	1.8

The accuracy of the developed method was determined by comparing the results of experiments repeated 9 times in three series in samples with 10%: 20%: 30% more than the nominal value

Table 5. – Determining the accuracy of the equipment

Amlodipine drawer, g	**Amount added, g**	**Total amount, g**	**Determined amount, g**	**Determined amount,%**
0.001 0.001 0.001	0.0001 0.0001 0.0001	0.0011 0.0011 0.0011	0.0009 0.0009 0.0009	82 82 100 } 88
0.001 0.001 0.001	0.0002 0.0002 0.0002	0.0012 0.0012 0.0012	0.0011 0.0010 0.0012	92 83 100 } 92.6
0.001 0.001 0.001	0.0003 0.0003 0.0003	0.0013 0.0013 0.0013	0.0011 0.00128 0.0013	85 98.5 100 } 94.5

Average value 91.7%; The range of values is 88–94.5%

Conclusion

1. A moderate method of extracting amlodipine from biological fluid was developed. The most suitable extractant was found to be chloroform;

2. The isolated amlodipine was identified using chromatographic and physical methods;

3. The absorption pathways of the IR–spectrum of amlodipine were determined;

4. IR–spectroscopic method of quantitative analysis for amlodipine was developed;

5. The method was validated according to parameters such as precision, accuracy, reproducibility, linearity.

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