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CHEMICAL-TOXICOLOGICAL STUDIES OF INDAPAMIDE

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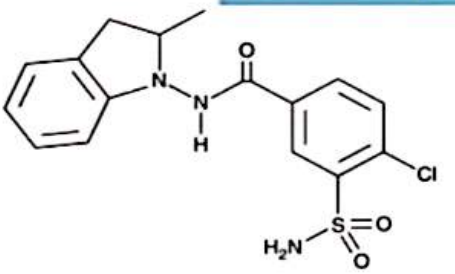
ABSTRACT

"The class of diuretic drugs includes torasemide, furosemide, indapamide, dichlothiazide, polythiazide, mannitol, and others. Indapamide is a diuretic drug belonging to the group of non-thiazide sulfonamide derivatives and contains an indole ring. In terms of its pharmacological properties, it is closely related to thiazide diuretics. Like thiazide diuretics, it acts on the proximal part of the distal convoluted tubules of the nephron, where it induces an increase in the secretion of sodium and chlorides, and to a lesser extent, potassium and magnesium, thereby increasing the volume of excreted urine [1]. Phase II and III clinical trials have demonstrated that the hypotensive effect of indapamide is maintained for 24 hours. This effect is observed even when administered in doses that induce only a mild diuretic effect. The antihypertensive properties of indapamide are associated with an improvement in the elasticity of arterial walls and a reduction in arterial resistance, as well as total peripheral vascular resistance. Indapamide also reduces left ventricular hypertrophy [2]. For thiazides and thiazide-like diuretics, the dosage is strictly defined; exceeding it does not enhance the therapeutic effect but instead increases the occurrence of side effects. Therefore, if the current treatment proves ineffective, the dosage of the drug should not be increased. However, in some instances, patients may increase the dose without a physician's prescription, which can lead to adverse effects or toxicity [3]."

Keywords: Indapamide, chemical-toxicological analysis, biofluid, model object, blood, urine

Purpose of the scientific work: Development of modern analytical conditions for the drug substance Indapamide and its application to model biological objects and fluids.

General properties of indapamide



Chemical name: 3- (aminosulfonyl) -4-chloro-N- (2,3-dihydro-2-methyl-1H -indol-1) benzamide, International name: Indapamide, Brutto-formula: $C_{16}H_{16}ClN_3O_3S$, Molecular weight: 365.83, melting point: 160-162°, Description: White crystalline powder, readily soluble in methanol, acetic acid, ethyl acetate, slightly soluble in chloroform. Dosage form: tablet. Synonyms: Arifon, Indapen, Indamed, Indapafon, Indipam

Physical properties: Maximum light absorption by UV spectrophotometry (methanol): 242, 278, 286 nm. Dissolves well in methanol, acetic acid, ethyl acetate, slightly in chloroform. Melting point 160-162°C. Hypertensive agent; diuretic, Therapeutic doses and method of administration. Therapeutic dose 2.5mg/day. It has been established that up to a dose of 40 mg does not have a toxic effect. This indicates 16 times the therapeutic dose. LD 50 in mice, rats, guinea pigs (mg/kg): 393-421, 410-564, 347-416..

For the determination of indapamide, the methods of thin-layer chromatography (TLC), spectrophotometry (SP) were used..

Methods and styles:

Thin-layer chromatography method. In order to determine the authenticity of the standard substance Indapamide by the TLC method, a mixture of organic solvents and opening reagents was selected. For the selection of opening reagents, 0.01 g (precise weight) of the standard substance indapamide was taken, placed in a 100 ml volumetric flask, and dissolved in 95% ethyl alcohol. From this standard solution, using a calibrated capillary tube, 25 µl were dropped in a circle with a width of 5 mm at a distance of 2 cm from each other onto a chromatographic plate coated with silica gel, pre-prepared in laboratory conditions. The plate was dried at room temperature (18-20°C) and sprayed with stain-forming reagents with various chemical properties. Of the reagents used: a yellowish spot on UV light, yellow with 50% HNO3 and Marky reagent, then pink, dark pink with Erdman reagent, light brown to dark brown with Mandelin reagent and FPN

reagent (5% $FeCL_3$, 20%HCl, 50% HNO_3 The appearance of yellow, pink spots was observed as a result of standing with a reagent consisting of a mixture of reagents[4].

At the next stage of the research, the distribution of indapamide in a mixture of organic solvents was studied. The analysis was carried out on chromatographic plates coated with silica gel, prepared under laboratory conditions. The plates were dripped onto the starting line with a standard solution of indapamide, then dried at room temperature and immersed in a chromatographic chamber pre-saturated with the mixture of solvents. When the solvents on the chromatogram rose from the starting line to the finish line, the plates were removed from the chamber, dried at room temperature, and sprayed with one of the above reagents to determine the point of accumulation of the substance along the plates. In this case, the Rf values of the resulting spots were determined.

Results: The results of the analysis are presented in Table 1..

1-table

Results of the selection of a mixture of organic solvents used in the analysis of indapamide by the TLC method

№	Organic solvent mixture	Rf value of indapamide
1	Benzene: Acetone (80:20)	0,43-0,45
2	Benzene: dioxane: ammonia (60:35:5)	0,67-0,69
3	Chloroform: Acetone (18:1)	0,60-0,62
4	Dioxane:toluene (10:3)	0,80-0,82
5	Propanol:benzene:toluene (10:5:2.5)	0,88-0,90
6	Benzene:dichloroethane:propanol (10:7:3)	0,85-0,87
7	Dichloroethane: dioxane: ammonia (8:4:1)	0,36-0,38
8	Propanol: dioxane: ammonia (7:4: 0.5)	0,77-0,79
9	Chloroform: dichloroethane: ammonia (8:7:1)	0,44-0,46
10	Benzene: propanol: ammonia (7:7:0.5)	0,88-0,90
11	Chloroform: benzene: ammonia (6:5:0.5)	0,65-0,67
12	toluene: dioxane: ammonia (10:4:1)	0,55-0,57
13	Benzene:dichloroethane (7:2.5)	0,20-0,22

From the results of the analysis, it became known that from the mixture of organic solvents, a mixture of organic solvents containing: benzene: dioxane: ammonia (60:35:5) Rf=0.67 was taken as moderate. Of these, a mixture of chloroform:acetone (18:1) organic solvents was chosen as the working solvent system in chemical-toxicological analyses.

In chromatographic analyses, it is important to determine the reaction sensitivity of reagents that detect the test substance for this substance. In this regard, the sensitivity and specificity of the above-recommended analysis methods for indapamide were studied. To determine the location of indapamide spots on the chromatogram, the reagents used above were used, and their sensitivity to the substance was studied. For this purpose, a series of working standard solutions with a decreasing concentration were prepared from the solution of the standard indapamide sample, which were dropped in a circle 0.4-0.5 cm wide at a distance of 2 cm from each other on the starting line of the chromatographic plate using a microspray, and their chromatographic distribution was carried out in a mixture of organic solvents containing chloroform: acetone (18:1). Then it was sprayed with the recommended chemical reagents. The results of the analysis are presented in Table 2.

Table 2.

Results of determining the sensitivity of indapamide to stain-forming reagents by the TLC method..

Reaction sensitivity, μg	Stain-forming reagents					
	UV rays	HNO	Marquis reagent	Erdman's reagent	Mandeline reagent	5% $FeCL_3$ 20%HCl 50% HNO_3
10	+	+	+	+	+	+
9	+	+	+	+	+	+
8	+	+	+	+	+	+
7	+	+	+	+	+	+
6	+	+	+	+	+	+
5	+	+	+	+	+	+
4	+	+	-	+	+	+

3	+	+	-	+	+	+
2	+	+	-	-	-	+
1	+	+	-	-	-	-
0,5	+	+	-	-	-	-

As a result of the experiments, it was established that among the reagents used to detect indapamide, the sensitivity of 50% nitric acid and UV radiation to the substance is 0.5 μg.

At the next stage of the experiment, the influence of sorbents on the TLC analysis of indapamide was studied. For this purpose, silica gel plates prepared in laboratory conditions, ready-made chromatographic plates containing the sorbent "Silufol UV-254" and "Aluminum oxide" were used. Silica gel chromatographic plates, prepared under laboratory conditions, were dried first at room temperature, then at a temperature of 105C for 30 minutes in a drying oven.. The prepared silica gel plates were stored vertically in a special container-exsicator until analysis. To perform the analysis, 0.1 ml of a 95% ethyl alcohol solution containing 1 μg/ml of the standard substance indapamide was added to the starting line of the plates and dried at room temperature. Then the plates were immersed in chromatographic chambers filled with a chloroform:acetone (18:1) mixture and saturated with their vapors, and the chromatographic process was carried out. To determine the location of the substance's accumulation on the chromatographic plate, UV light was used, followed by a 50% HNO3 solution. The results of the analysis are presented in Table 3.

3-table

Results of the study of the influence of sorbents on the analysis of indapamide by the TLC method.

Selected systems	Plays used		
	"Silufol UV-254"	Aluminum oxide	Silica gel
Benzene:dioxane:ammonia (60:35:5)	0,63-0,65	0,67-0,69	0,67-0,69

Chloroform:acetone (18:1)	0,40-0,42	0,66-0,68	0,60-0,62
Chloroform:Benzene:Ammonia (6:5:0.5)	0,21-0,32	0,80-0,82	0,65-0,67

The analysis results showed that it is advisable to use chromatographic plates containing the sorbent "Aluminiy oksid" and "Silikagel," prepared under laboratory conditions.

When analyzing complex mixtures by the TLC method, it is important to study the conditions characteristic of the tested substance. The fact that the results of the conducted experiments are specific to the tested substance and that other compounds do not interfere with the test results is also of great importance in the analyses. In this case, stain-forming reagents should be sprayed onto the substance being tested on the chromatographic plate, and the resulting stain should be distinguished by its color and Rf value.. In this regard, the study of the specificity of the proposed analytical conditions for indapamide is one of the important tasks [5].

To study the specificity of the selected analytical conditions for the determination of indapamide by thin-layer chromatography, solutions of standard samples of some antiarrhythmic drugs in 95% ethyl alcohol (2.5 mg/ml) were used. Chromatographic plates with solutions of various antiarrhythmic drugs were analyzed under TLC conditions in the above-recommended system and Rf values were determined. The results of the analysis are presented in Table 4.

4-table

On the study of indapamide specificity by the TLC method obtained results

Name of articles	Plays used		
	"Silufol UV-254"	"Aluminum oxide"	Silica gel
Indapamide	0,63	0,67	0,67
Berlipril	0,21	0,13	0,21
Enalapril	0,10	0,23	0,12

As can be seen from the data in Table 4, when analyzed using the proposed TLC method, other drugs differ from each other in Pf indicators and do not interfere with the determination of indapamide.

Development of conditions for the analysis of indapamide by UV-spectrophotometric method. It was confirmed that the solution of indapamide in 96% ethyl alcohol has a high absorption index at a wavelength of 243 nm.

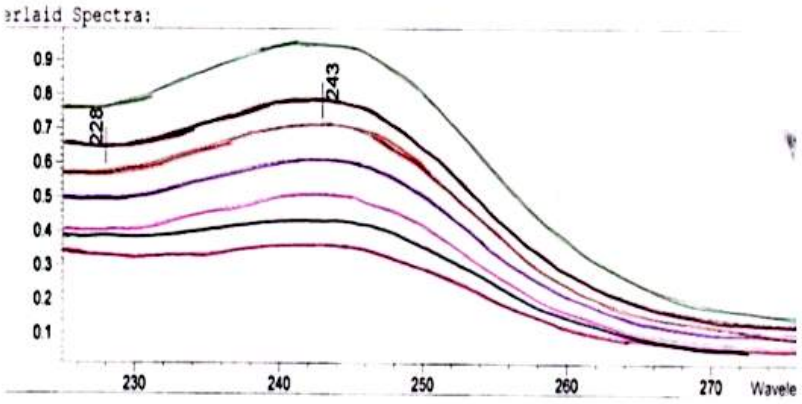


Figure 1. Radiation absorption indicators of standard working solutions of indapamide

Quantitative analysis of indapamide by UV-spectrophotometric method was calculated using a calibrated diagram. For this purpose, working standard solutions of indapamide containing 1-10 μg/ml were prepared from the above-prepared solution B and analyzed in a cuvette with a layer thickness of 10 mm at a wavelength of 243 nm. 95% ethyl alcohol was used as the reference solution (Fig. 3).)

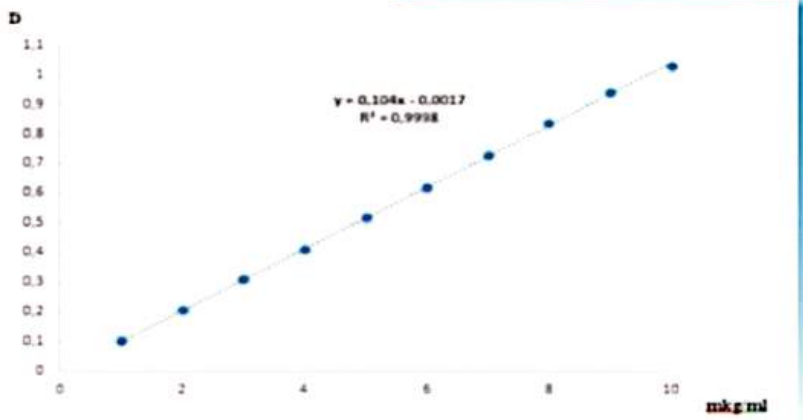


Figure 2. Diagram of the dependence of the optical density of indapamide on concentration

Based on the obtained results, the values of the molar light absorption index of indapamide and are calculated. The results of the performed analysis are presented in Table 5 [6]..

5-table

Comparative and molar light absorption indicators of indapamide detection results (n=5)

Substance ntent, mcg/ml	Optical density (D)	Specific light absorption index (E)	Molar light orption readings ()
1	0,103	103	3765
2	0,208	104	3801
3	0,309	103	3764
4	0,410	100	3655
5	0,518	104	3787
6	0,620	103	3776
7	0,728	104	3801
8	0,835	104	3801
9	0,940	104	3816
10	1,030	103	3765

Average		103	3774

Then, a quantitative analysis of indapamide was carried out to verify the accuracy and reproducibility of the analytical conditions developed by the spectrophotometric method. For this purpose, 5 samples were prepared from a 7 μg/ml indapamide solution, and the optical density of the solutions was determined on a spectrophotometer at a wavelength of 243 nm. Based on the compiled calibration scheme, the amount of indapamide was determined, and the metrological report was calculated according to the XI edition of the State Pharmacopoeia. The results of the analysis are presented in Table 6.

6-Table

Results of UV-spectrophotometric analysis of indapamide content

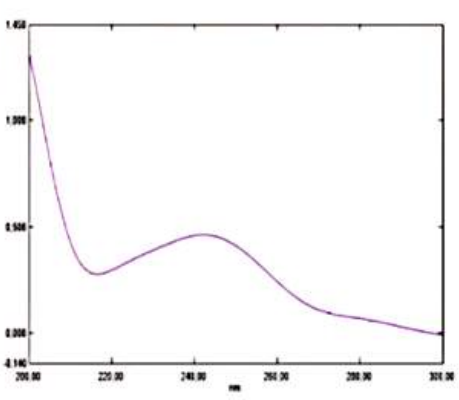
Amount of drug mcg/ml	Amount found		Metrological characteristic	
	mkg/ml	%		
7,0	7,02	100,3	$\overline{X}$ =100,04	S^2=0,113
7,0	6,98	99,8	S =0,336	S_x=0,150
7,0	7,02	100,4	ΔX=1,96	$\Delta\overline{X}$ = 0,877
7,0	6,97	99,6	ε =1,962%	$\bar{\varepsilon}$=0,877
7,0	7,01	100,1		

As can be seen from Table 6, as a result of spectrophotometric analysis of indapamide, an average of 100.04% was obtained. In this case, the average relative error was 0.877%. The obtained results show that the developed method can be used to determine the amount of indapamide isolated from biological objects and biological fluids.

The analysis of indapamide by UV-spectrophotometric method was studied. In this case, it was established that indapamide solution in alcohol has a maximum light absorption at a wavelength of 243 nm. The linearity, accuracy, and reproducibility of the method relative to the substance were studied. Quantitative analysis of indapamide by UV-spectrophotometric method was calculated using the compiled calibration scheme, in which the specific and molar light absorption indicators of indapamide were 103 and 3774, respectively [7].

Isolation of indapamide from biological fluids. 10 ml of urine and 5 ml of blood samples (1 ml of a solution containing 1 mg of standard indapamide solution) were brought to a pH of 4.0-4.5 with a 0.1 N sulfuric acid solution, to which 2 ml of a 25% ammonium sulfate solution and 10 ml of ethyl acetate organic solvent were added and shaken in a mechanical shaker for 10 minutes. After this, it was centrifuged for 5 minutes (3000 rpm) to precipitate the protein substances in the mixture. The organic layer was separated from the aqueous layer, the remaining aqueous layer was extracted 5 times with 10 ml of ethyl acetate, and the organic layer was poured out. Ethyl acetate extracts were combined, passed through filter paper containing 5 g of anhydrous sodium sulfate salt, and evaporated until a dry residue remained.. The residues were dissolved in 5 ml of 95% ethyl alcohol and then purified from foreign substances using the method of thin-layer chromatography of indapamide[7].

Cleaning from foreign substances by the TLC analysis method. For this purpose, a drop of the obtained alcoholic solution in the form of a line is applied to the starting line of chromatographic plates prepared in laboratory conditions, and a working standard solution of indapamide is added to one side as a confirmer and dried at room temperature. The plates were immersed in a chromatographic chamber filled with a mixture of organic solvents chloroform: benzene: ammonia (6:5:0.5) and saturated with their vapors, and the mixture of solvents was raised to a height of 10 cm.. Upon reaching the finish line, the plates were removed and dried at room temperature. To determine the location of the elevated accumulation of the substance on the chromatographic plates, the side of the sorbent to be scraped was covered, and the opening reagent with 50% nitric acid was sprayed onto the part where indapamide was dripped. The part of indapamide with pink spots was marked correctly, the sorbent layers were scraped off, and the sorbents were placed in separate porcelain dishes. They were dissolved in 5 ml of ethyl alcohol, filtered into a 10 ml volumetric flask, and brought to the line with 95% ethyl alcohol[8]. From this solution, indapamide was analyzed by spectrophotometric and HPLC methods under the above-mentioned conditions.



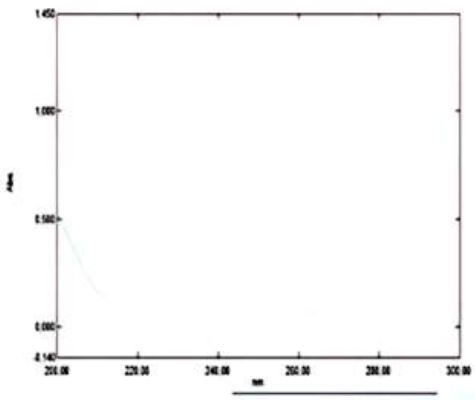


Figure 3. Isolated from urine spectrum of indapamide

Figure 4. Isolated from blood spectrum of indapamide.

Table 7

Results of indapamide urinary excretion

1.0 mg of substance added to 10 ml of urine

UV-spectrophotometry method		
determined quantity		metrological analysis
мг	%	results
0,717 0,704 0,711 0,718 0,7247	71,79 70,45 71,12 71,85 72,42	$\overline{X}$ =71,53 S^2=0,574 S=0,758 S_x=0,339 ΔX=2,103 $\Delta\overline{X}$ =0,941 ε =2,94% $\overline{\varepsilon}$ =1,32%

8-table

Results of indapamide isolation from blood

1.0 mg added to 5 ml of blood

UV-spectrophotometry method		
determined quantity		metrological analysis results
mg	%	
0,60 0,59 0,58 0,60 0,61	60,14 59,86 58,45 60,99 61,22	$\overline{X}$ =60,11 S^2=1,153 S =1,073 S_x=1,073 ΔX=1,333 $\Delta\overline{X}$ = 1,333 ε =1,331% $\overline{\varepsilon}$=2,215%

As can be seen from the data presented in the table, indapamide isolated under the recommended extraction conditions was determined by UV spectrophotometry in an average of 71.53% in urine and 60.11% in blood.

These results showed that the proposed methods can be used in cases of poisoning with indapamide in isolation from biological objects and biological fluids (blood, urine)..

Conclusion:

1. In the analysis of indapamide by the thin-layer chromatography method, a mixture of solvents in the ratio benzene: dioxane: ammonia (60:35:5) from the system of organic solvents used in the analysis by the thin-layer chromatography method was found to be appropriate. In it, it was possible to confirm indapamide's Rf in the range of 0.67-0.69. UV glow and 50% nitric acid were found to be the most sensitive in spot illumination. Its sensitivity to the substance was 0.5 μg.

2. The conditions for quantitative analysis of indapamide by UV-spectrophotometry were studied. At high wavelengths of light absorption in the UV region, the average values of the specific and molar light absorption indicators averaged 103 and 3774,

respectively. Under analytical conditions, the linearity range of indapamide was 1-10 µg/ml.

3. Conditions for the analysis of indapamide by high-performance liquid chromatography were developed. The retention time of indapamide under the developed conditions was 6.041. The linearity and sensitivity of the analysis conditions were studied. As a result of the experiments, it was established that the linear range of the method for indapamide is 0.04-1.0 µg and the sensitivity is 0.01 µg. The amount of indapamide determined by high-performance liquid chromatography was 98.7%.

4. Methods for isolating indapamide from biological objects and biological fluids have been developed.

5.In the recommended extraction conditions, indapamide was isolated by spectrophotometry in the amount of 71.53% in urine and 60.11% in blood.

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