Original Article

SIOFOR DRUG ANALYSIS FOR THE FORENSIC CHEMISTRY PRACTICE

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Abstract

Siofor (metformin hydrochloride) is widely used to maintain weight in the second type of diabetes and as a complex treatment of obesity, as well as a slimming agent in biologically active supplements under various names. The presence of metformin hydrochloride with the strong effect of reducing sibutramine in Reduksin Forte capsules, taking it together with other disproportionate drugs causes an increase in cases of acute poisoning. In such cases, in order to provide quick medical assistance to patients, in order to obtain quick and accurate results in forensic chemistry practice, specific chemical toxicological analysis conditions are needed. The results of the analysis of Siofor (metformin hydrochloride) presented in this scientific article by thin layer chromatography and UV-spectrophotometry methods are closely related to forensic chemistry practice.

Keywords: Siofor, analysis, thin layer chromatography, UV-spectrophotometry, metformin hydrochloride.

INTRODUCTION

Siofor (metformin hydrochloride) is a drug that is used to reduce blood sugar and the long-term complications of diabetes. The antidiabetic drug siofor is the main treatment regimen for type 2 diabetes and is also widely used for weight loss in obese patients. This is a long-term drug that helps to gradually reduce excess body fat. Metformin hydrochloride is recommended to be used in addition to a diet, in which the amount of calories consumed is gradually reduced.

In recent years, the increase in obesity among the population has increased the demand for siofor, and articles about the results of metformin in medical journals have led to the widespread acceptance of this drug among the people. In rare cases, taking Siofor together with disproportionate drugs can lead to poisoning.

Scientists have found out that 40% of experimental rats died when the drugs chloroquine and hydroxychloroquine, used against COVID-19, were given together with metformin.

When the bodies of dead rats were dissected, death was observed in all of them due to an increase in the number of autophagosome cells in the heart, liver, kidney, and brain. Based on the conducted experiments, scientists emphasize that patients with diabetes who take metformin hydrochloride should be selected with extreme caution when suffering from coronavirus.

In addition, patients taking Siofor are asked to eat grapefruit or citrus fruits with caution. If Siofor is taken in the morning, citrus fruits in the evening, or vice versa. When a patient treated with Siofor takes alcohol, it causes a sharp drop in blood sugar and diabetic coma. One of the side effects of the drug Siofor is the risk of developing lactic acidosis accompanied by hypoxia. Lactic acidosis is a rare but potentially fatal complication [1,4,8,9].

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Purpose of work

Analysis of Siofor drug for forensic chemistry practice by thin layer chromatography and UV-spectrophotometry methods was taken as a goal.

Methods and materials

Information about the analysis of Siofor by the TLCh method is given in the literature. However, in the available analyses, it was found that the sensitivity and specificity of the method for the drug siofor was studied very little. Based on the above, it was considered one of the important tasks to develop the analysis conditions of sioforin by the TLCh method. In this regard, existing methods were improved and new convenient, sensitive and moderate conditions were developed in order to develop the conditions for the analysis of sioforin TLCh.

Silica gel and calcium sulfate reagents were selected for the preparation of plates for the experiment. Glass plates measuring 9 x 12 cm were used for this. For the preparation of gypsum-silica gel plates, a total of 35 g of silica gel, 2 g of gypsum and 90 ml of purified water are used for 10 plates of 9 x 12 cm size, but it should also be said that silica gel, gypsum and purified water should be added to each plate separately. water was measured and prepared in a separate container. After thoroughly mixing gypsum and silica gel in a porcelain mortar, water is added and mixed until a uniform mass is formed, and then transferred to the surface of the plates. It is very important that the sorbent layers are arranged in the same way in the prepared plates. Then the prepared plates are dried in a drying cabinet at a temperature of 110 o C for 1 hour.

When methods with high sensitivity are used, even the smallest amount of a toxic substance in a biological object can be determined. After all, it is known that the residual amounts of toxic substances are very small in the tested items. For this reason, it is important to choose the reagents that identify the substance in chromatography and to determine its sensitivity and specificity for the same substance. Various chemical reagents were used in order to determine the place where the substance was collected in the chromatographic plate. During the selection of reagents, siofor was first dissolved. Metformin hydrochloride substance and ethyl alcohol were used in the preparation of Siofor solution (3.0 mg of metformin was dissolved in 1 ml of ethyl alcohol).

Research results

Several of the previously prepared plates were taken, their surface was divided into six cells, and a few drops of 1% metformin solution were dripped into each cell. Then, reaction experiments to metformin were conducted with the help of more than 20 different opening reagents, and the formation of spots with some chemical reagents was observed. Among them, dark golden color on a light yellow background with Bouchard's reagent, golden color on a yellow background with Dragendorf's reagent, dark brown on a yellow background with a solution of 1% iodine in 2% KJ, blue on a pink background with cobalt rhodanide reagent. color, iron II sulfate, Mandelin reagent gave dark brown spots on a yellow background.

After that, reactions of metformin aqueous solution with selected reagents were observed in a test tube. As a result, it was observed that metformin, like on the KSK plate, formed a brown precipitate with Dragendorff, Bouchard, Mandelin, iron II sulfate solution, and a yellow precipitate with bromine water.

So, Dragendorff, Lugol, cobalt rhodanide, Mandelin, Bouchard reagents were found to be the opening reagents. The results of the analysis are presented in Table 1 below.

Table 1 Results of chemical reactions of metformin on a chromatographic plate with chemical reagents

Reagent name	Result	Reagent name	Result
Bouchard's reagent	A brown spot on a yellow background	Feling II solution	The color did not change
10% solution of potassium permanganate	The color did not change	Dragendorf’s reagent	The color did not change
5% solution of ammonium molybdate	The color did not change	Zonnenshtey n’s reagent	The color did not change
Lugol's solution	A brown spot on a yellow background `	Xlor-ruh-yod reaktivi	The color did not change
Frede's reagent	The color did not change	10% solution of sodium hydroxide	The color did not change
Rahmatov’s reagent	The color did not change	Ultra violet illumination light	No gleam
Cobalt rhodanide solution	Blue color on a pink background	40% solution of iron sulfate	The color did not change
Bromine water solution	Orange spot on light yellow background	Feling I solution	The color did not change

Mandelin reagent	A brown spot on a yellow background	Picric acid	The color did not change

In order to determine the level of sensitivity of the opening reagents, a series of working standard solutions with decreasing concentration of 1% solution of metformin was prepared. Samples from these solutions were dripped onto the chromatographic plate and glassware in the form of circles 0.4-0.5 cm wide at a distance of 2 cm from each other using a microsyringe or a marked glass capillary tube. The plate was dried at room temperature, and the reagents selected for analysis were sprayed and dripped. As a result of the reactions, Lugol's reagent revealed sensitivity to 0.0025 mg or 2.5 μg of metformin. The results are presented in Figure 1 and Table 2.

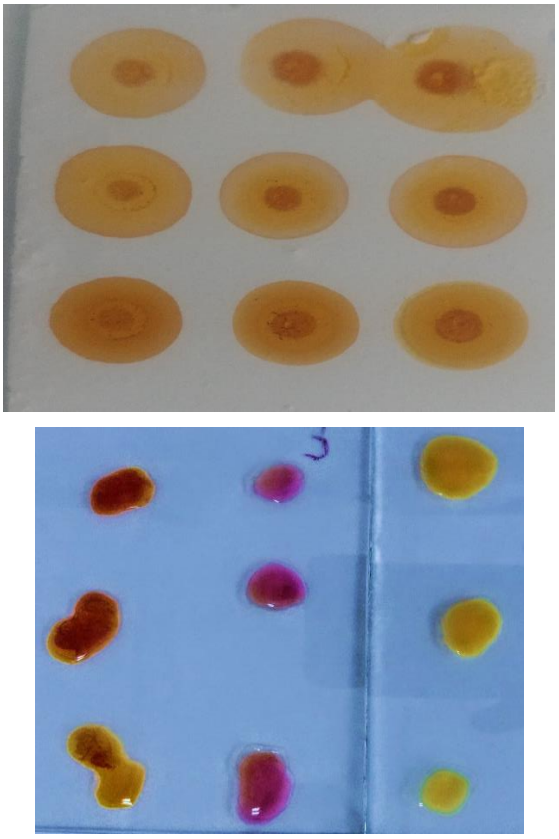

Figure 1. Determination of reaction sensitivity using reagents

Table 2 The results of determining the sensitivity of reagents that form spots in the analysis of metformin by thin layer chromatography method

Amount of metformin taken for analysis, mkg	Stain-forming reagents		
	Dragendorf	Bushard	Lyugol
5,0	+	+	+
4,5	+	+	+
4,0	+	+	+
3,5	+	+	+
3,0	+	+	+
2,5	-	-	+
2,0	-	-	-

Note: "+" - positive, "-" - negative results.

Among these reagents, Lugol's reagent was found to have a very high reaction sensitivity for metformin.

Thin layer chromatography is based on separation of substances in a mixture due to different distribution in a thin layer of a mobile eluent (mixture of solvents) and a sorbent uniformly attached to the plate. The correct choice of solvent system plays an important role in the distribution and separation of substances in thin layer chromatography. A properly selected mixture of organic solvents is not only an indicator of quality (by Rf value) for this substance, but also plays an important role in the removal of foreign substances [2,5,6]. Also, for the practice of forensic chemistry, when substances are analyzed by TLCh method, their Rf values are required to be in the range of 0.4 - 0.7, and this is achieved by using organic solvents in different proportions.

At the next stage of the work, the distribution of metformin in various organic solvents and their mixtures was investigated. The main attention was paid to the polarity and harmlessness of organic solvents and to the physico-chemical properties of the tested substance.

Experiments for the analysis of metformin by TLCh method were carried out on ready-made "Silufol UB 254" and "KSK" silica gel chromatographic plates, which are widely used in forensic chemistry and chemical toxicological laboratories. A chromatographic plate containing silica gel (containing 13% gypsum) was prepared according to the procedure described in the literature. Chromatographic plates are dried in a drying cabinet at 110 oC for 1 hour. The prepared silica gel plates were kept in special containers - desiccators until analysis. Several chromatographic chambers were taken to perform the analysis. A 5-7 mm thick mixture (system) of solvents acting as a mobile phase prepared for analysis is poured under the vessel. In order for the chromatographic chambers to be well saturated with organic solvents, strips of filter paper were hung on its walls. The lower end of the strips was immersed in an organic solvent. This situation serves to prevent some negative factors (the effect of exclusion, the entering of stains into an uncertain form, etc.). The mouth of the chamber is closed and left to saturate for 15-20 minutes. Saturating the chamber with solvent vapors helps to distribute the test mixture correctly and qualitatively to certain components.

The starting line was drawn with a soft pencil at a distance of 1.5 cm from the lower edge of the prepared plates. Small drops of the standard solution of metformin, the detectable substance, are dripped using a thin capillary tube at a distance of 0.5 cm from the start line. The solvent from the plate is dried at room temperature. Metformin-dropped chromatographic plates are quickly lowered into chambers

saturated with organic solvent vapors, and the solvent mixture is left to reach the finish line. The chromatographed plates are removed from the chamber and dried at room temperature. Then it is sprayed with one of the reagents listed above, Lugol, Bouchard, Dragendorff. The Rf values of the spots formed by metformin in organic solvent mixtures were determined.

The correct choice of sorbent and mobile phase for the thin layer chromatography method is of great importance for the correct performance of the analysis. We used mixtures of several organic solvents in different proportions as mobile phase. The results of the analysis are presented in Table 3 below.

Table 3 Organic solvent system and results used in the analysis of metformin by thin layer chromatography

№	System of organic solvents	Rf indicator	
		KSK plate	Silofol plate
1	Benzene:Dioxane:Ammonium hydroxide:(60:35:5)	0	0
2	Benzene: Acetone (80:20)	0,1	0,2
3	Xloroform:Atseton (90:10)	0	0
4	Dioxane: Chloroform (50:50)	0	0
5	Chloroform: Ethanol (7:3)	0	0
6	Acetone: Chloroform: 25% ammonia: Dioxane (5:45:2,5:47,5)	0	0
7	Chloroform: Formic acid: Ethanol (8:1:1)	0,08-0,10	0,10-0,13
8	Benzene: Butanol (9:1)	0,10-0,12	0,11-0,14
9	Butanol:Acetic acid:Water (6:4:2)	0,65-0,67	0,67-0,69
10	Ethyl acetate: Acetone: Diethylamine (2,5:2,5:5)	0	0
11	Benzene: Ethanol (5:5)	0,03-0,05	0,06-0,09
12	Acetone: Water (7:3)	0,25-0,28	0,27-0,30
13	Dioxane: toluene: 25% ammonia (3:6:1)	0,73-0,75	0,76-0,79
14	Butanol: Acetone (3:7)	0	0
15	Chloroform:Dioxane (8:2)	0	0
16	Butanol-1:vinegar:water:acetone (5:3:1,5:1)	0,35-0,37	0,36-0,38
17	Propanol: vinegar: water: acetone (5:3:1,5:1)	0,52-0,54	0,54-0,56
18	Ethanol: vinegar: water: acetone (5:3:1,5:1)	0,56-0,58	0,58-0,60
19	Ethanol: vinegar: water: benzene (5:3:1,5:1)	0,56-0,58	0,58-0,60

Table 3 presents the results of the chromatographic process carried out using mixtures of some organic solvents in different proportions on chromatographic plates made of "Silufol UB 254" and "KSK" silica gel (containing 13% gypsum).

Based on the conducted experiments, the average Rf in organic solvent systems of metformin organic solvents ethanol:acetic acid:water:acetone (5:3:1.5:1) and ethanol:acetic acid:water:benzene (5:3:1.5:1) At the value of =0.56-0.60, a clear, small spot was observed.

It is important to study the specifics of the analysis conditions selected for the substance under investigation by the TLCh method. It is of great importance in the analyzes that the conditions for the determination of these compounds with a complex composition are specific to the tested substance and that other compounds do not interfere with the test results. In this case, the tested substance on the chromatographic plate should be distinguished from other compounds by the color and Rf value of the spot formed when the illuminating reagents are sprayed. After all, similar Rf values and spot colors can lead to wrong conclusions.

The many use of Bouchard, Lugol, Dragendorf reagents in chemical-toxicological analyzes also requires conducting these experiments. In this regard, one of the important tasks is to study the specificity of the proposed analysis conditions for metformin. To perform this task, solutions of standard samples of other anti-obesity drugs used together with metformin were used. Since sibutramine is most often used in complex with siofor, chromatographic plates, on which solutions of sibutramine samples were dropped, were analyzed under TLCh conditions recommended above and Rf values were determined. The results are presented in Table 4.

Table 4 The results of the study the specificity of the developed TLCh analysis conditions for metformin

Test substance	Rf value		Spot color	
	ethanol:vinegar:water:benzene (5:3:1,5:1)	**anol:vinegar:water: acetone (5:3:1,5:1)**	**lugol**	**Dragendorf's reagent**
Reduxin (sibutramine)	Rf=0,72-0,76	Rf=0,72-0,76	brown spot on a yellow background	golden spot on a yellow background
Siofor (metformin)	Rf=0,56-0,60	Rf=0,56-0,60	brown spot on a yellow background	golden spot on a yellow background

As can be seen from the data in Table 4, the proposed TLCh differs in terms of Rf values of antiobesity reducer samples in the analysis conditions and does not interfere with the detection of siofor. The obtained results suggest the use of siofor extracted from the composition of biological objects and biological fluids in the practice of forensic chemistry for cleaning from foreign substances.

Taking into account that the UV-spectrophotometric method is widely used in daily analysis, it is quick and easy to perform experiments, and almost all laboratories are equipped with this instrument, it was considered appropriate to develop it as an alternative to the HPLC method. After all, the sensitivity of the method allows it to analyze the extracts extracted from the composition of the biological object, after certain purifications [3,7].

The SHIMADZU 1800 spectrophotometer was used to develop the UV-spectrophotometric analysis of Sioforn.

To determine the optical density of metformin, its standard solution in water was analyzed in a cuvette with a layer thickness of 10 mm and a wavelength of 190 to 350 nm. Purified water was used as a reference solution. It was determined that the solution of metformin in water has a high light absorption index at a wavelength of lmax=233 nm. The results of the analysis are presented in Figure 2.

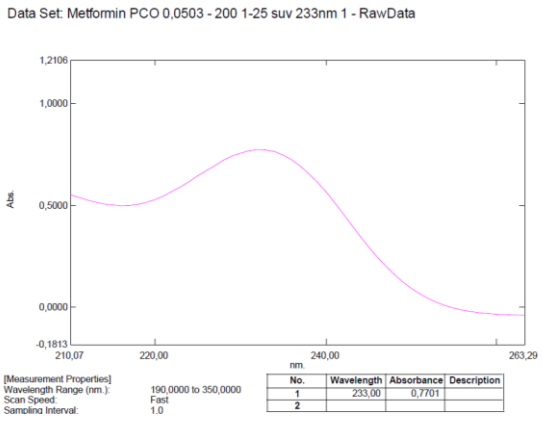

Figure 2. Spectrum of metformin determined by UV spectrophotometry

Based on the results of the experiment, it was aimed to carry out an analysis by the UV-spectrophotometry method for Siofor containing metformin. For this, 10 tablets of Siofor were taken from the drug and mixed together. A mass of powder weighing 1 tablet of the mixed powder was placed in a 50 ml bottle. It was poured with purified water. Placed in auto mixer. After the mass in the flask was dissolved, it was brought up to the mark with purified water. The solution was filtered. Siofor test solution was analyzed in a cuvette with a layer thickness of 10 mm, wavelength from 190 to 350 nm. Purified water was used as a reference solution. It was determined that the solution of siofor in water has a high light absorption index at a wavelength of λmax=233 nm. The result of the analysis was found to be the same as the results of the metformin standard solution (Figure 3).

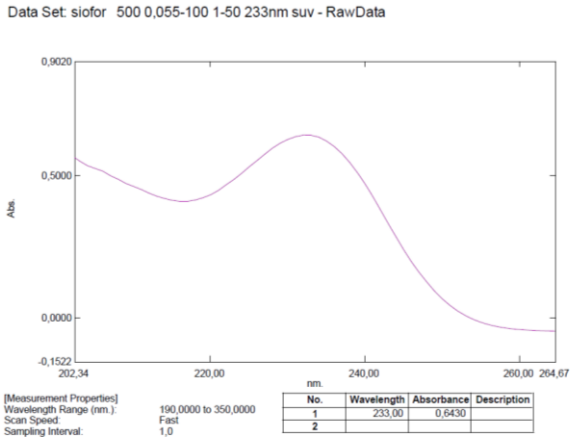

Figure 3. The spectrum of Siofor (metformin) determined by UV spectrophotometry

At the next stage of our research, we aimed to study the quantitative analysis of metformin.

Quantitative analysis of metformin by UV spectrophotometry was calculated using a calibration plot. For this, 0.0134 (exact draw) metformin was weighed, placed in a 50 ml measuring flask and dissolved with purified water. The prepared solution was brought up to the mark of the measuring flask with water, and working standard solutions of different concentrations were prepared from this solution. A calibration plot was drawn based on the optical density of the prepared working standard solutions (Figure 4). Based on the results of the

analysis, it was determined that the solution of metformin in the amount of 2-16 µg/ml according to the Bouguer-Lambert-Behr law.

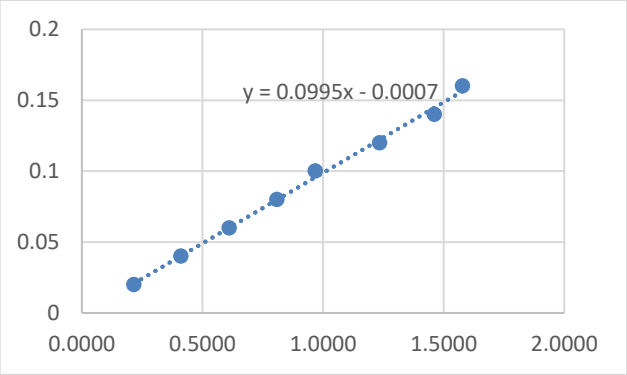

Figure 4. Graph of dependence of concentration of working standard solution of metformin on optical density.

Determination of the amount of metformin in micrograms of the tested solutions is carried out on the basis of the following formula.

$$X = \frac{D \cdot V \cdot 100}{E_{1cm}^{1\%} \cdot 100 \cdot V_1}$$

In this,

D - optical density of the solution;

$E_{1cm}^{1\%}$ - relative absorption index of sibutramine;

V – the volume of the tested solution, ml;

V1 – the volume of the tested object, ml.

In order to determine the sensitivity of this analytical method for siofor, working standard solutions with decreasing concentrations of metformin 1% standard solution in water were prepared and their optical densities were determined at a wavelength of 233 nm. Based on the obtained results, the sensitivity of the UV-spectrophotometry method for siofor was 2 µg/ml.

Based on the data obtained on the basis of the analysis, the relative light absorption index values of siofor were calculated. The results of the performed analysis are presented in Table 5.

Table 5 The results of determination of indicators of comparative and molar absorption of metformin

№	The amount of substance taken for the experiment		Optical densityD	Comparative light absorption index $E^{1\%}_{1см}$	Molar absorption index
	%	µg/ml			
1	0,02	2	0,2157	107,850	17863,196
2	0,04	4	0,4110	102,750	17018,483
3	0,06	6	0,6113	101,883	16874,937
4	0,08	8	0,8084	101,050	16736,912
5	0,1	10	0,9684	96,840	16039,609
6	0,12	12	1,2351	102,925	17047,468
7	0,14	14	1,4621	104,436	17297,687
8	0,16	16	1,5781	98,631	16336,294
	average			101,887	16770,268

As can be seen from the results of the analysis in Table 5, the relative and molar light absorption indicators of siofor were on average 101.887 and 16770.268 respectively.

Then a quantitative analysis was carried out in order to check the accuracy and reproducibility of the analysis conditions developed by the spectrophotometric method for siofor. For this, 5 samples of 10 µg/ml solution of siofor were prepared, and the optical density of the solutions was determined in a spectrophotometer at a wavelength of 233 nm. The amount of siofor was determined on the basis of the created calibration chart, and the metrological report was calculated according to the DF XI publication. The results of the analysis are presented in Table 6.

Table 6 Results of the analysis of the amount of Siofor by ultraviolet spectrophotometric method

The amount of the drug, mg/ml	Amount found		Metrological description
	mg	%	
500	512,1	102,4	X_{aver}=100,4 S^2=l,49 S =1,22 S_x=0,54 ΔX=2,798 $\Delta X_{ўр}$= 1,251 E=7,10% $E_{ўр}$=3,17%
500	501,7	100,3	
500	495,5	99,1	
500	499,6	99,9	
500	503,0	100,4	

As can be seen from Table 6, as a result of the spectrophotometric analysis of siofor, the average value was 100.4%. In this case, the average relative hardness was 3.17%. The obtained results show that the obtained results can be used to determine the amount of siofor in the practice of forensic chemistry.

Conclusions

1) Lugol's, Bouchard's and Dragendorff's reagents were found to be the most suitable as opening reagents for Siofor (metformin). 2) Lugol's solution was found to have a very high reaction sensitivity for metformin among the opening reagents. 3) Based on the conducted experiments, metformin organic solvents are used in organic solvent systems in the ratio of ethanol:acetic acid:water:acetone (5:3:1.5:1) and ethanol:acetic acid:water:benzene (5:3:1.5:1). a clear, small spot was observed at the average value of Rf=0.56-0.60. 4) Under the proposed TLCh analysis conditions, solutions of other anti-obesity drug samples differ in terms of Rf values and do not interfere with the determination of metformin. 5) The conditions of analysis of metformin and siofor by UV-spectrophotometry were studied. It was confirmed that metformin and siofor solutions in water have a high light absorption index at a wavelength of lmax=233 nm.

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