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THE STUDY OF CLONIDINE BY METHOD OF THIN LAYER CHROMATOGRAPHY

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Annotation. Studies of clonidine by thin layer chromatography were carried out using modern equipment for TLC analysis (highly sensitive and selective chromatographic plates, organic solvent systems and universal developers).

It has been established that systems of mobile solvents and chromatographic plates are the most suitable for identification and purification from impurities for the analysis of biological objects on clonidine: chloroform-methanol (90:10) - ($R_{f\ clonidine}$ = 0.50±0.02); methanol-n-butanol (60:40) - ($R_{f\ clonidine}$ = 0.61±0.02) (Sorbfil PTLC - AF-A).

Key words: Clonidine, thin layer chromatography (TLC) method.

Introduction. Clonidine – N–(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine hydrochloride is the antihypertensive drug. 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 [1,2]. Clonidine also causes a decrease in intraocular pressure, associated with a decrease in secretion and improved outflow of aqueous humor. Due to its effect on the central nervous system, the drug has an analgesic and sedative effects [3,4].

Clonidine in overdose and self-medication can cause severe intoxication [5,6]. Clonidine is known in criminal practice as a drug that is used by criminals to add to alcohol and put the victim to sleep for robbery and murder [7].

Among the modern methods of analysis to create a database of parameter identification of analytes in biological objects TLC method is one of the most suitable methods for sensitivity and selectivity.

The earlier developed methodology of TLC analysis of clonidine using a variety of different chromatographic conditions (chromatographic plates, organic solvents systems and developers), which are based on the individual properties of the drug [2,7]. Considering the use mixtures of drugs for the treatment and combined intoxications actual problem of chemical-toxicological analysis is the use of unified TLC method suitable for solving practical problems of healthcare.

The development of new and modification and improvement of existing chromatographic techniques for the study of clonidine, suitable for chemical-toxicological analysis, is an actual task.

Purpose of work – the selection of optimal TLC conditions for the analysis of biological objects for clonidine using modern highly sensitive and selective chromatographic plates, organic solvents systems and universal developers.

Materials and methods of research. The choice of optimal conditions for TLC chromatography of clonidine was carried out on several different chromatographic plates, which were widely used in modern chemical-toxicological studies [8]:

- **A** - *Sorbfil PTLC -AF-A* (type of sorbent - silica TLC -1A, graining - 5-17 microns, thickness - 110 mm, a binding agent – silicasol, type bases - aluminum foil, plates size – 10 x10 cm);
- **B** - *Sorbfil PTLC-P-B-UV* (type of sorbent - silica TLC -1B, graining - 8-12 microns, thickness - 100 mm, a binding agent – silicasol, type bases - PETF-E (Polyethylene and Teflon), plates size – 10 x10 cm).

Organic solvents corresponded to the qualification of "PFA": chloroform, acetone, ethylacetate, methanol, n-butanol, dioxane, toluene, benzene (Sigma-Aldrich, USA). Reagents corresponded to the qualification of "PFA": 25% solution of ammonium hydroxide (Chimmed, Moscow, Russia).

Chromatographic studies of clonidine were performed by the method of ascending, one-dimensional thin-layer chromatography. The chromatographic system of organic solvents (50.0 ml) was introduced into a chromatographic chamber (glass vessel with volume of 500 cm^3), carefully was closed the chamber with a lid, followed by saturation of the chamber with solvent vapors at least 30-60 minutes.

Chromatographic plates were cut into strips of a certain width in accordance with the size of the chamber. The working methanol solutions of clonidine with a concentration of 20.0 μg / ml were used in the study on the basis of the Kharkiv Regional Bureau of Forensic Medicine. These solutions were applied to the point using a calibrated capillary on the start line of the chromatographic plate at a distance of 1-2 cm from the edge. The diameter of the spot was 0.3-0.5 cm.

The chromatographic plate was placed in a chromatographic chamber. The length of the run of the front of the mobile phase was 7 cm. Chromatography was completed when the solvent reached the finish line. The chromatographic plate was dried at room temperature, then identification of the test substance was performed using the most sensitive location reagents at R_f values.

The identification of clonidine was performed by comparing the spots on the chromatogram obtained for the test solution, with the corresponding spot on the chromatogram obtained for the standard sample (solution for comparison). The comparison was made by staining, size and value retention - R_f for both spots, where R_f - ratio of the distance the substance travels from origin to the distance the solvent travels from origin [2].

It was found that one of the most sensitive location reagent for clonidine is reagent of Dragendorff in the modification of Mounier gave orange color of spots, the sensitivity was 1.0 μg in the sample.

Chromatographic behavior of clonidine was investigated by TLC in 10 solvent systems, including:

- **systems,** which are recognized standard by the International Committee for systematic toxicological analysis of the International Association of Forensic Toxicologists - 1) chloroform-methanol (90:10), 2) ethylacetate-methanol-25% solution of ammonium hydroxide (85:10:5), 3) methanol, 4) methanol-n-butanol (60:40), 5) methanol-25% solution of ammonium hydroxide (100:1.5); 6) acetone;
- **systems,** which are used in general organic TLC screening substances - 7) chloroform-dioxane-acetone-25% solution of ammonium hydroxide (47.5:45:5:2.5), 8) toluene-acetone-ethanol-25% solution of ammonium hydroxide (45:45:7.5:2.5), 9) ethylacetate-methanol - 25% solution of ammonium hydroxide (85:10:2.5), 10) chloroform-n-butanol-25% solution of ammonium hydroxide (70:40: 5) [2].

Results and discussion. As a result of TLC investigation were established the most optimal conditions for the preliminary and confirmatory studies of clonidine and for the identification and purification of the test substance in the presence of biogenic impurities (Table).

Table

The R_f value of clonidine for the various types of chromatographic plates in systems of organic solvents ($n = 5$)

The R_f value of clonidine	System	The R_f value of clonidine	System
Sorbfil PTLC -AF-A			
0.50±0.02	1	0.75±0.03	6
0.86±0.03	2	0.75±0.03	7
0.71±0.02	3	0.70±0.02	8
0.61±0.02	4	0.84±0.03	9
0.76±0.03	5	0.69±0.02	10
Sorbfil PTLC-P-B-UV			
0.65±0.02	2	0.81±0.03	8
0.74±0.03	7	0.79±0.03	9

As a result of TLC-studies in systems (1-10) the most effective conditions were established (system of mobile solvents - chromatographic plates) for identification and purification of clonidine:

- chloroform-methanol (90:10) - A ($R_{f\ clonidine}$ = 0.50±0.02);
- methanol-n-butanol (60:40) - A ($R_{f\ clonidine}$ = 0.61±0.02).

The results of TLC investigation of clonidine are intended for employees of the Bureau of Forensic Medical Examination, toxicological and narcological centers, clinical laboratories for the study of medicinal substances in biological objects.

Conclusions:

1. For chemical-toxicological analysis of clonidine are recommended the most effective conditions (system of mobile solvents - chromatographic plates): chloroform-methanol (90:10) - ($R_{f\ clonidine}$ = 0.50±0.02); methanol-n-butanol (60:40) - ($R_{f\ clonidine}$ = 0.61±0.02) (Sorbfil PTLC -AF-A).

2. The results of TLC investigation of clonidine are intended for employees of the Bureau of Forensic Medical Examination, toxicological and narcological centers, clinical laboratories for the study of medicinal substances in biological objects.

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