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THE USE OF TLC- METHOD FOR CHEMICAL-TOXICOLOGICAL ANALYSIS OF TERAZOSIN

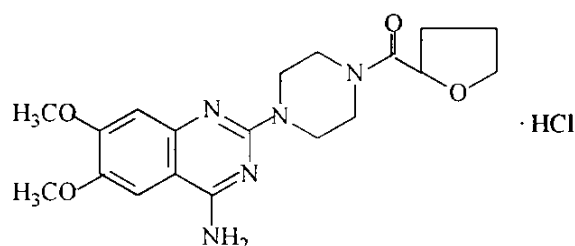
Annotation. The choice optimal conditions of analysis of terazosin hydrochloride by Thin-Layer Chromathography — method (systems of organic solvents, stationary! phase, location reagents), suitable for chemical-toxicological investigations has been conducted.

The results ofTLC-analysis may be recommended for direct investigations of biological material on terazosin.

Key words: terazosin hydrochloride, Thin-Layer Chromathography — method.

I. Introduction

Terazosin hydrochloride - (RS)-6,7-dimethoxy-2-[4-(tetrahydrofuran-2-yl-carbonyl)-piperazin-1-yl]quinazolin-4-amine hydrochloride.



The drug is used for treatment of hypertension and benign prostatic hyperplasia [1]. The cardiovascular system is amazed with an overdose or self-medication of terazosin, the activity of the CNS is inhibited, respiratory system is broken [1-4], because the choice of highly sensitive and selective methods of study of terazosin hydrochloride in biological objects is an important issue. In carrying out the modern chemical-toxicological analysis of drugs are widely used chromatographic methods (high performance liquid chromatography (HPLC), gas liquid chromatography (GLC), thin layer chromatography (TLC) techniques and spectral (UV spectrophotometry, mass spectrophotometry) [5-7].

TLC-method is characterized by high sensitivity, selectivity, simplicity and accessibility to the experimental technique, which leads to its use for screening of toxic substances, cleaning substances from biogenic impurities, identification and quantitative determination [2].

Purpose of work – choice of optimal conditions of analysis of terazosin hydrochloride by TLC-method, suitable for chemical and toxicological studies.

II. Raising of task

TLC analysis of terazosin was carried out by ascending, dimensional thin layer chromatography. For selecting optimal chromatographic conditions of terazosin as thin layers of adsorbents used chromatographic plates, which are widely used in studies of biological objects:

► **A – Sorbfil PSTH-AF-A** (type of sorbent – silica STH-1A, graining – 5-17 microns, thickness – 110 mm, a binding agent – silicasol, type bases – aluminum foil, plates size – 10x10 cm);

► **B – Sorbfil PSTH-P-B-UV** (type of sorbent – silica STH-1B, graining – 8-12 microns, thickness – 100 mm, a binding agent – silicasol, type bases – PETF-E (Polyethylene and Teflon), plates size – 10x10 cm);

► **C – Glass plates by «Merck» (Germany)** (type of sorbent – silica gel 60 F254, graining – 10-12 microns, type basis – glass plates size – 10x20 cm).

Technique of definition sensitivity of the location reagents with terazosin TLC analysis: to determine the sensitivity of terazosin hydrochloride 0,05 g were added to a volumetric flask 1000,0 ml, dissolved in 96% ethanol and the solution is brought up to the mark with ethanol (standard solution, 50 µg / ml). In a series of volumetric flasks were added 100,0 ml from burette 1,0; 2,0; 4,0; 6,0; 8,0; 10,0; 12,0; 14,0; 16,0; 18,0 and 20,0 ml of the standard solution and the solutions are brought up to the mark with ethanol (solution 1 with a concentration – 0,5 µg / ml, solutions 2 – 11 with concentrations 1,0 – 10,0 µg / ml, respectively). The sensitivity of the location reagents was determined using multiple solutions obtained after appropriate dilution of the standard solution and the chromatogram development in organic solvent system with R_f terazosin = 0,4-0,6. For studies were used chromatographic Sorbfil plates PSTH-P-V-UV and the organic solvent – methanol. The results are shown in Table 1.

Table 1 Sensitivity location reagents of terazosin in TLC analysis ($n = 5$)

| Reagent | Staining spots | Sensitivity, µg |
|---|----------------|-----------------|
| UV light ($\lambda = 254$ nm) | Purple | 0,5 |
| Dragendorff reagent for Mounier | Orange | 1,0 |
| Iodine vapor | Orange | 3,0 |
| Reaction to the primary aromatic aminogroup | Purple | 5,0 |

Chromatographic behavior of terazosin was investigated by TLC in 16 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-acetone (80:20), 2) ethylacetate, 3) chloroform-methanol (90:10), 4) ethylacetate-methanol-25% ammonia solution (85:10:5), 5) methanol, 6) acetone, 7) methanol-25% ammonia solution (100:1.5), 8) methanol- n-butanol (60:40), 9) cyclohexane-toluene-diethylamine (75:15:10);

► **Systems**, which are used in general organic TLC screening substances – 10) chloroform-acetone-dioxane-25% ammonia solution (47,5:45:5:2,5), 11) toluene-acetone-ethanol-25% ammonia solution (45:45:7,5:2,5), 12) ethylacetate-methanol – 25% ammonia solution (85:10:2,5), 13) chloroform-n-butanol-25% ammonia solution (70:40 : 5);

► **Systems**, which are proposed for the analysis of a quinazoline derivatives – 14) ethylacetate-methanol-hexane (80:10:10), 15) ethylacetate-hexane-methanol – 25% ammonia solution (45:45:5:5), 16) acetone-toluene – 25% ammonia solution (6:4:1) [7].

TLC analysis was performed according to the procedure: at the start line of the chromatographic plate by a distance of 1-2 cm from the edge at a point applied with the calibration capillary 20,0-50,0 µg of study medication using its 0,01% alcoholic solution. The spot diameter should be less than 0,5 cm.

Chromatography was performed in a chamber volume of 500 cm³, into which 50,0 ml of an appropriate solvent system were added with subsequent saturation of the chamber solvent vapors at least 30 minutes; path length of the front of the mobile phase – 7 cm. Chromatography was terminated when the solvent reached the finish line. Chromatographic plate was dried at room temperature, after which identification was carried out by using UV light and Dragendorff's reagent for Mounier (Table 1.).

Terazosin spots were identified by comparing the chromatogram obtained for the sample solution with a corresponding spot on the chromatogram obtained for a reference sample (reference solution). The comparison was made by staining, size and value retention – R_f for both spots where R_f – ratio of the distance between the point of application and the center of the spot to the distance traveled by the solvent front [2].

III. Results

As a result of TLC studies were established the most optimal conditions for the identification and purification of terazosin in the presence of biogenic impurities:

► solvent systems – acetone or toluene-acetone-ethanol-25% ammonia solution (45:45:7,5:2, 5);

► chromatographic plates – Sorbfil PSTH-AF-A, Sorbfil PSTH-P-B-UV or glass plates by «Merck» ($R_{f \text{ terazosin}} = 0,50-0,55$) (Table 2).

Table 2 R_f values of terazosin for the various types of chromatographic plates in solvent systems ($n = 5$)

| System | Types of chromatographic plates | | | System | Types of chromatographic plates | | |
|--------|---------------------------------|------|------|--------|---------------------------------|------|------|
| | A | B | C | | A | B | C |
| 1 | 0,06 | 0,30 | 0,06 | 9 | 0,48 | 0,55 | 0,48 |
| 2 | 0,07 | 0,11 | 0,04 | 10 | 0,55 | 0,55 | 0,31 |
| 3 | 0,51 | 0,63 | 0,48 | 11 | 0,53 | 0,54 | 0,55 |
| 4 | 0,68 | 0,63 | 0,53 | 12 | 0,52 | 0,50 | 0,40 |
| 5 | 0,62 | 0,54 | 0,65 | 13 | 0,51 | 0,79 | 0,54 |
| 6 | 0,50 | 0,53 | 0,50 | 14 | 0,35 | 0,41 | 0,15 |
| 7 | 0,56 | 0,86 | 0,60 | 15 | 0,75 | 0,88 | 0,81 |
| 8 | 0 | 0 | 0,85 | 16 | 0,31 | 0,38 | 0,35 |

The results of TLC analysis may be recommended for directional investigations of biological material on terazosin.

Conclusions

For directional chemical-toxicological analysis of terazosin are recommended:

- solvent system – acetone or toluene-acetone-ethanol-25% ammonia solution (45:45:7,5:2,5);
- chromatographic plates – Sorbfil PSTH-AF-A, Sorbfil PSTH-P-B-UV or glass plates by «Merck» ($R_{f\text{ terazosin}} = 0,50-0,55$).

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