THE CHOICE OF OPTIMAL CONDITIONS FOR CHEMICAL-TOXICOLOGICAL ANALYSIS OF CLEMASTINE BY THIN-LAYER CHROMATOGRAPHY

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Introduction. Clemastine hydrofumarate (Tavegil) – 1-methyl-2 [2-α-methyl-p-chlorobenzhydryloxy)-ethyl]-pyrrolidine fumarate. Tavegil is a highly effective antihistamine drug, similar to the action of dimedrol. The drug has a high anticholinergic activity, but to a lesser extent penetrates across the blood-brain barrier. The drug exists in an injectable form, which can be used as an additional agent for anaphylactic shock and angioedema, for the prevention and treatment of allergic and pseudo-allergic reactions. However, hypersensitivity to clemastine and other antihistamines, which have a chemical structure similar to them, is known.

Clemastine in overdose and self-treatment affects the central nervous system and causes severe intoxication, because the choice of highly sensitive and selective methods of study of Clemastine in biological objects is an important issue. In carrying out the modern chemical-toxicological analysis of drugs are widely used thin-layer chromatography-method (TLC), which leads to its use for screening of toxic substances, cleaning substances from biogenic impurities, identification and quantitative determination.

Aim. The choice of optimal conditions of analysis of Clemastine by TLC-method, suitable for chemical and toxicological studies.

Materials and method. TLC analysis of Clemastine was carried out by ascending, dimensional thin layer chromatography. For selecting optimal chromatographic conditions of Clemastine as thin layers of adsorbents used chromatographic plates, which are widely used in studies of biological objects: 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);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);Glass plates by "Merck" (Germany) (type of sorbent – silica gel 60 F_{254}, graining – 10-12 microns, type basis – glass plates size – 10 x 20 cm).

Chromatographic behavior of Clemastine was investigated by TLC in 8 solvent systems, which are recognized standard by the International Committee for systematic toxicological analysis of the International Association of Forensic Toxicologists – chloroform-acetone (80:20), ethylacetate, chloroform-methanol (90:10), ethylacetate-methanol-25% ammonia solution (85:10:5), methanol, acetone, methanol-25% ammonia solution (100:1,5), methanol-n-butanol (60:40).

For selecting optimal chromatographic conditions of Clemastine were studied 4 solvent systems, which are used in general organic TLC screening substances – chloroform-acetone-dioxane-25% ammonia solution (47,5:45:5:2,5), toluene-acetone-ethanol-25% ammonia solution (45:45:7,5:2,5), ethylacetate-methanol – 25% ammonia solution (85:10:2,5), chloroform-n-butanol-25% ammonia solution (70:40:5).

It was found that the most sensitive location reagents for Clemastine are UV light (λ = 254 nm). The spots were painted in violet color, the sensitivity was 0,5 – 1,0 μg in the sample. Reagent Dragendorff in the modification of Mounier gave orange color of spots, the sensitivity was 1,0-3,0 μg in the sample.

Results and discussion. As a result of TLC studies were established the most optimal conditions for the identification and purification of Clemastine in the presence of biogenic impurities: solvent systems – ethylacetate – methanol -25% solution of ammonium hydroxide (85:10:5**)**; chromatographic plates – Sorbfil PTLC -AF-A ($R_{f\ \text{Clemastine}}$ = 0,45±0,03).

The results of TLC analysis may be recommended for directional investigations of biological material on Clemastine for preliminary studies – solvent systems – methanol – 25% solution of ammonium hydroxide (100:1,5); chromatographic plates – Sorbfil PTLC -AF-A ($R_{f\ \text{Clemastine}}$ = 0,60±0,03); Sorbfil PTLC-P-B-UV ($R_{f\ \text{Clemastine}}$ = 0,43±0,03).

In screening studies combined poisonings is recommended to use the system – toluene – acetone – ethanol – 25% solution of ammonium hydroxide (45:45:7,5:2,5) and chromatographic plates – Sorbfil PTLC -AF-A ($R_{f\ Clemastine}$ = 0,55±0,03).

Conclusions. The choice of optimal conditions of analysis of Clemastine by TLC–method (systems of organic solvents, stationary phase, location reagents), suitable for chemical-toxicological investigations has been conducted.

For directional chemical-toxicological analysis of Clemastine are recommended: stationary phase – Sorbfil PTLC -AF-A, the systems of organic solvents – ethylacetate – methanol -25% solution of ammonium hydroxide (85:10:5**)** (R_f = 0,45±0,03). The location reagents of Clemastine – UV light and Dragendorff's reagent for Mounier.

VARIATION IN TERPENE LACTONES COMPOSITION IN *GINKGO BILOBA* L. LEAVES DUE TO THE GROWTH LOCATION AND TIME OF HARVEST

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Introduction. The standardization of *Ginkgo biloba* leaf extract, which is used in the production of medicines, is problematic due to the insufficiently revealed relation between accumulated compounds and a therapeutic response. The composition of the primary plant material becomes significant in the production of non-standardized phyto-pharmaceutical products. Pharmaceutical companies prefer using cultivated plants instead of wild-harvested plants because they show smaller variation in their constituents. Furthermore, when medicinal plants are produced by cultivation, the main secondary metabolites can be monitored, permitting the definition of the best period for harvesting. Research in changing quantities of bioactive compounds of the plants that are grown in specific production areas is applied in order to reduce variation in the possible composition and the therapeutic response. The content of bioactive compounds differs during the plant growth period and can be influenced by geographical location, soil type, cultivation methods, natural biological variation and season, time of harvesting, drying and processing techniques as well as storage conditions. Thus, it is important to study the seasonal differences in the contents of active compounds.

Aim. To investigate the qualitative and quantitative composition of terpene lactones in leaf samples of *G. biloba* growing in Lithuania. Also to present a recommendations for the industry for rational planning of the collection of plant material rich in terpene lactones.

Materials and methods. Different age *Ginkgo biloba* phenotypes from four different collections (the northern and western parts of Lithuania) were studied. The sample size was 40 leaves in each sample collected from all directions of the tree. Leaves collected from tree were mixed to get a representative sample. The temperature and rainfall during the vegetative period was close to the multi-annual average. There were no extremes that could affect normal development of *G. biloba* trees.

1.5 g of leaves was combined with 7.5 mL of MeOH: H_2O mixture (1:1, *V*/*V*) and kept in an ultrasound bath for 5 min. The remaining residue was poured into 7.5 mL of MeOH: 2M HCl mixture (1:1, *V*/*V*), mixed thoroughly and kept in ultrasonic bath for 5 min. After removal of the top fraction of the solution, the extraction was repeated by adding 7.5 mL of MeOH: 2M HCl mixture (1:1, *V*/*V*). All fractions were collected in a 25 mL flask, and the content was made up with MeOH: H_2O mixture (1:1, *V*/*V*) to the mark. The resulting solution (10 mL) was transferred to a 10 mL bottle and heated in a boiling water bath (100±2°C) for 2.5 h.

The HPLC equipment system consisted of a Waters 2695 chromatograph that was equipped with a Waters 2998 DAD and **Waters 2424 ELSD detectors.** The chromatographic separation was carried out using a 250×4.6 mm, 5 µm ACE 8 column that was thermostated at 25°C. Elution method- Isocratic. Mobile phase- tetrahydrofuran: methanol: water (10:20:70, *V*/*V*/*V*). Flow rate -1.0 mL min^{-1}, injection volume – 10 µL. The drift tube and nebulizer temperatures for ELSD were set at 80°C and 30°C, resp.