**Aim.** To develop sensitive and specific methods for milnacipran detection and quantitative determination using thin layer chromatography (TLC) and UV-spectrophotometry.

**Materials and methods.** $R_f$ values of milnacipran in ten mobile phases including those which are recommended by The International Association of Forensic Toxicologists for TLC drug screening for four types of chromatographic plates (Sorbfil, Merk, plates manufactured in Estonia with KSKG sorbent, Silufol UV-254,) were determined. The UV-spectrum of milnacipran in methanol was measured over 215–380 nm wavelength range. Stock solution (SS) (2000 µg/ml) and 10 working standard solutions (WSS) (60.0; 200.0; 300.0; 400.0; 600.0; 800.0; 1000.0; 1200.0; 1400.0 and 1500.0 µg/ml) of the drug were prepared.

**Results and discussion.** Three mobile phases of methanol–25% ammonia (100:1.5) ($R_f$=0.37±0.04), ethyl acetate–methanol–25% ammonia (85:10:5) ($R_f$=0.53±0.04) and ethyl acetate–acetone–25% ammonia (50:45:4) ($R_f$=0.81±0.05) had a low correlation of $R_f$ values (they are given for Sorbfil plates). Absorption maxima were detected at wavelengths of 256±2, 262±2, 267±2 and 272±2 254±2 nm. The calibration curve was described by the following equation: y=0.000640x+0.029 (r=0.9994), LOD and LOQ values were of 16.9 µg/ml and 51.0 µg/ml, respectively. The linearity of the calibration curve was within the range of milnacipran concentrations from 60.0 to 1500 µg/ml.

**Conclusions.** The developed methods of milnacipran detection and quantitative determination using TLC and UV spectrophotometry are sensitive and selective enough for chemical-toxicological analysis.

# RESEARCH OF CHROMATOGRAPHICAL PARAMETERS OF LAMOTRIGINE FOR THE PURPOSES OF CHEMICAL-TOXICOLOGICAL ANALYSIS

Voronovich A. S.
Scientific supervisors: prof. Merzlikin S. I., assoc. prof. Stepanenko V. I.
National University of Pharmacy, Kharkiv, Ukraine
voronovichandrey@gmail.com

**Introduction.** According to epidemiological services, more than 40 million people suffer from epilepsy worldwide. This ailment is annually detected in 40-70 people per 100 thousand population, and the incidence rates reach 3%. In Ukraine, there are currently about 100,000 patients diagnosed with epilepsy and 500,000 people with its manifestations.

For the treatment of epilepsy, a wide group of anticonvulsants is used. Lamotrigine is one of the most common medicines in this group of drugs. But, according to the analysis of FDA and patientsville.com websites it has been found that more than 30 countries have reported lethal poisonings with lamotrigine.

**Aim** The aim of this work is to study the chromatographic parameters of lamotrigine in a thin layer of sorbent for the purposes of chemical toxicological analysis.

**Materials and methods.** As biological objects, model samples of fresh porcine liver weighing 20 g, which have been used saturated with 10 mg of lamotrigine.

Isolation of lamotrigine from the biological matrix was performed with acetonitrile, acidified with HCL. For this, 20 g of ground pork liver, pre-saturated for 24 h with ethanolic solution of lamotrigine (10 mg), was placed in a flask, added 50 ml of acetonitrile, acidified by 6 M. HCL solution to pH 2.0-2.5, infused for 30 min and filtered. The resulting extract was basified with 30% NaOH to pH = 9 and extracted with chloroform. The resulting extract was evaporated to dryness, dissolved in 10 ml of ethanol and examined.

Studies were performed on chromatographic plates Merck silica gel 60 $F_{254}$ (made in Germany) of dimensions 10 × 10 cm. Before elution of the samples, the chromatographic plates were pre-impregnated with methanol and activated in a drying Cabinet at a temperature of 110-120 $^0$C for 0.5 h.

The following solvent systems were used as mobile phases: butanol-glacial acetic acid -water (30: 5: 15); ethyl acetate-methanol-25% ammonia solution (17: 2: 1); chloroform-n-butanol-25% ammonia solution (70: 40: 5); chloroform-methanol (9: 1); methanol-25% ammonia solution (100: 1.5); butanol-glacial acetic acid-water (15: 5: 30); methanol-n-butanol (60:40); chloroform-ethanol (20: 1); ethyl acetate-chloroform-water (9: 3: 2.5).

*Chromatography technique.* The standard chromatographic camera was pre-saturated with eluent vapour for 30 min. 2 ml of chloroform extracts of lamotrigine are evaporated to a minimum volume of 0.05 ml. On the start line of the pre-activated chromatographic plate with a glass capillary, apply 5 μl of the corresponding extracts of lamotrigine. At a distance 1 cm apply 5 μl of the tested solutions of lamotrigine (5 μg in the sample) and caffeine (10 μg in the sample). The plate is placed in a chamber with a suitable solvent mixture and eluted. When the solvent front passes 8 cm from the start line, the plate is removed from the chamber, air-dried and sprayed with a Dragendorf reagent.

**Result and discussion.** Analysis of proposed lamotrigine detection and identification conditions in a thin layer of sorbent was performed using extractions obtained from liver tissues with acidified acetonitrile. In order to check the suitability of the chromatographic systems, the chromatography of the obtained chloroform extracts was carried out in parallel with the ethanol solution of caffeine. To visualize the adsorption zones of the test substances, the Dragendorf reagent was used as a common reagent. To visualize the caffeine stain, the corresponding strip was additionally sprayed with 10% sulphate acid. The chromatographic system is considered valid if the chromatogram clearly shows the caffeine spot.

The results of the studies are shown in the table. The study has shown that lamotrigine in all solvent systems used exhibits chromatographic mobility in the range of values $R_f$ from 0.25 ±0.02 to 0.88 ±0.02. However, the most suitable in the aspect of analytical diagnosis of acute lamotrigine poisoning has been found to be butanol-glacial acetic acid-water (30: 5: 15) system with $R_f$ value of the tested toxicant 0.43 ±0.02 and ethyl acetate-methanol-25% ammonia solution (17: 2: 1) with $R_f$ value 0.58 ±0.01.

For the detection of lamotrigine in biological objects, studies were conducted on the search for chromogenic reagents, such as: Erdmann's, Mandelin's, Frede, Marquis reagents, diphenylcarbazone solution in chloroform + 5% mercury (II) sulfate solution, potassium hexacyanoferrate (II) solution.

The most sensitive in terms of analytical diagnosis of lamotrigine poisoning were Erdmann's and Mandelin's reagents (brown and yellow-brown coloration, respectively). The detection sensitivity of 0.5 μg in the sample, both when carrying out reactions in thin layers of sorbent, and when carrying out reactions with dry residues of the toxicant. These reagents can be used in the preliminary stage of chemical toxicology analysis. However, they are not specific.

**Table. Chromatographic mobility parameters of lamotrigine**

| No No. | Chromatographic system | $R_f$ value of test substances | |
|---|---|---|---|
| | | Lamotrigine | Caffeine |
| 1 | Butanol -glacial acetic acid -Water (30: 5: 15) | 0.43 ±0.02 | 0.31 ±0.02 |
| 2 | Ethyl acetate-methanol-25% ammonia solution (17: 2: 1) | 0.58 ±0.01 | 0.45 ±0.02 |
| 3 | Chloroform-n-butanol-25% ammonia solution (70: 40: 5) | 0.60 ±0.02 | 0.42 ±0.01 |
| 4 | Chloroform-methanol (9: 1) | 0.33 ±0.02 | 0.24 ±0.02 |
| 5 | Methanol-25% ammonia solution (100: 1,5) | 0.70 ±0.01 | 0.43 ±0.02 |
| 6 | Butanol -glacial acetic acid -Water (15: 5: 30) | 0.66 ±0.02 | 0.45 ±0.01 |
| 7 | Methanol-n-butanol (60:40) | 0.25 ±0.02 | 0.13 ±0.01 |
| 8 | Chloroform-ethanol (20: 1) | 0.88 ±0.02 | 0.53 ±0.02 |
| 9 | Ethyl acetate-chloroform-water (9: 3: 2,5) | 0.81 ±0.01 | 0.46 ±0.02 |

**Conclusions.** Thus, the chromatographic mobility of lamotrigine in a thin layer of sorbent has been investigated. The conditions for chromatography and identification of the toxicant in biological objects that can be used in chemical-toxicological analysis for lamotrigine poisoning have been determined