APPLICATION OF TLC, HPLC AND GLC IN THE ANALYSIS OF METRONIDAZOLE AND SECNIDAZOLE

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Metronidazole and secnidazole are attributed to the group of antiprotozoal medicines and widely used for treatment of infectious diseases, at the same time they are possessed of quite a number of side effects showed by symptoms of acute intoxication, especially when interacting with alcohol.

The research purpose is to develop the conditions of metronidazole and secnidazole detection and identification by the methods of thin layer, high-performance liquid and gas-liquid chromatography when combined presence.

Metronidazole and secnidazole of pharmacopoeial purity were used in the experiment; their solutions in ethanol with the concentration of 1 mg/ml were prepared and gradually diluted in 10 – 10000 times.

Conditions of TLC-analysis: the chromatographic plates Sorbfil® PTLC-PH (silica gel STC-1HP, PETP, silica sol, 8 ÷ 12 μm fraction, 100 μm layer thickness) were used as the thin layers. The chromatographic dealing of metronidazole and secnidazole has been studied in 18 mobile phases: 1. chloroform – acetone (8:2); 2. ethyl acetate; 3. chloroform – methanol (9:1); 4. ethyl acetate – methanol – 25% NH_3 (85:10:5); 5. methanol; 6. methanol – *n*-butanol (6:4); 7. methanol – 25% NH_3 (100:1.5); 8. cyclohexane – toluene – diethylamine (75:15:10); 9. acetone; 10. chloroform – dioxane – acetone – 25% NH_3 (47.5:45:5:2.5); 11. toluene – acetone – ethanol – 25% NH_3 (45:45:7.5:2.5); 12. chloroform – *n*-butanol – 25% NH_3 (70:40:5); 13. chloroform; 14. chloroform – methanol – CH_3COOH cone. (90:10:1); 15. toluene – CH_3COOH cone. (3:1); 16. toluene – methanol – CH_3COOH cone. (9:1:1); 17. ethyl acetate – methanol – CH_3COOH cone. (85:10:2.5); 18. chloroform – methanol (1:1).

When using the mobile phases 3, 5, 8, 9 the investigations were carried out also at the plates processed previously with 0.1 mole/l KOH solution in methanol and then dried at 110°C for 30 min. In the mobile phase 6 the plates were previously processed with 0.1 mole/l NaBr solution.

UV-light before and after processing with the vapour of HCl; the Dragendorff reagent modified by Munier and 0.1 mole/l KOH solution in methanol were used for developing the spots of metronidazole and secnidazole at the plates.

Conditions of HPLC-analysis (the volume of injection – 100 μL): device – MiLiChrome® A-02; column – ∅2×75 mm, reversed phase ProntoSIL-120-5-C18 AQ; temperature – 40 °C; eluent A – 0.2 mole/l $LiClO_4$ – 0.005 mole/l $HClO_4$; eluent B – CH_3CN; flow – 100 μL/min.; gradient elution mode – linear from 5% to 100% CH_3CN for 40 min., then 100% CH_3CN for 3 min.; detector – UV-spectrophotometer (210, 220, 230, 240, 250, 260, 280, 300 nm).

Conditions of GLC-analysis (the volume of injection – 2 μL): device – HP 6890 Hewlett Packard; column – HP-1 ∅0.32 mm × 30 m, 0.25 μm, the thickness of layer of 100% dimethylpolysiloxane of 1 μm; temperature of the column thermostat – 70°C (3 min.), increasing the temperature with the rate of 40 °C/min. to 180 °C (keeping for 2 min.), increasing the temperature with the rate of 40°C/min. to 250°C (keeping for 3 min.); injector temperature – 280 °C; detector – flame-ionization; detector temperature – 280 °C; volume rate of carrier gas (helium) – 1.5 ml/min; stream dividing – 1:2.

The conditions of metronidazole and secnidazole detection and identification by the methods of thin layer, high-performance liquid and gas-liquid chromatography have been experimentally fitted.