The validation results obtained within one analytical run are presented in Table 1.

Table 1. The validation results of UV-spectrophotometric procedures of tinidazole, ornidazole and nimorazole quantitative determination in the variant of the method of additions

Parameter	Values			Acceptability criterion
	tinidazole	ornidazole	nimorazole	
stability				
$\delta^{model\ stability}$,%	1,37 (24 h)	0,95 (24 h)	0,68 (48 h)	$\leq 2.05\%$
linearity/calibration model				
b^{model}	0,980	1,006	0,959	–
s_b^{model}	0,005	0,015	0,004	–
a^{model}	1,068	0,079	2,695	$\leq 2.73\%$
s_a^{model}	0,542	1,650	0,485	$a^{model} \leq 2.015 \cdot s_a^{model}$
RSD_0^{model}	0,642	1,952	0,574	$\leq 3.18\%$
R_c^{model}	0,9999	0,9995	0,9999	≥ 0.9983
precision and accuracy				
$\overline{RR}^{model\ MA}$,%	101,24	99,43	102,39	–
$\delta^{model\ MA}$,%	1,24	0,57	2,39	$\leq 2.05\%$
$RSD_{RR}^{model\ MA}$,%	1,65	2,45	1,47	–
$\Delta_{RR}^{model\ MA}$,%	3,33	4,93	2,96	$\leq 6.40\%$

The total results of validation allow to point to the conclusion about acceptable stability, linearity, accuracy and precision of the developed UV-spectrophotometric procedures of tinidazole, ornidazole and nimorazole quantitative determination in the variant of the method of additions.

Conclusions. New procedures of tinidazole, ornidazole and nimorazole quantitative determination by the method of UV-spectrophotometry have been developed using 0.1 M HCl solution as a solvent; their acceptability for application in the variant of the method of additions has been shown.

APPLICATION OF THIN LAYER CHROMATOGRAPHY IN ANALYSIS OF EFAVIRENZ

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Introduction. HIV infection is usually treated with drug combinations consisting of at least three different antiretroviral medicines. Essential components of this highly active antiretroviral therapy are non-nucleoside reverse transcriptase inhibitors presented by five medicines (nevirapine, delavirdine, efavirenz, etravirine and rilpivirine). Efavirenz is recommended currently as preferred first-line medicine with a low pill burden, once daily dosing, a long half-life allowing for relatively stable plasma concentrations and some forgiveness for doses not taken exactly on schedule. There are cases of acute poisoning due to administration of efavirenz, including cases of suicide attempts. The method of thin layer chromatography (TLC) is widely used in the process of forensic toxicological examinations for screening and confirming investigations – with the purpose of analytes detection and identification respectively. The main focus is the chromatographic behaviour of the substances using standard mobile

phases (or solvents systems), as well as the conditions of analytes spots visualization using standard coloured reagents.

Aim. To carry out the integrated study of visualization conditions of efavirenz on TLC-plates with aplication of standard and particular coloured reagents, and also chromatographic behaviour of efavirenz using standard mobile phases.

Materials and methods. Efavirenz was of pharmacopoeial purity. The chromatographic plates Sorbfil® PTLC-IIH-UV and Merck® TLC Silica Gel 60 were used as thin layers. The colour reagents were prepared according to the Clarke's guide.

To choose the developing colour reagents in 10 μg of efavirenz were applied on the plates of both types, and then the reagents were sprayed or poured onto the plates. The results were fixed visually at once and after drying the plate, then the plates were developed in UV-light with the wavelength of 254 nm and 365 nm. At the next stage the plates were heated for 15 min. at 110°C (the plates were covered with glass plate), and then colours of spots were fixed in visible and UV-light one more time.

Chromatographing was carried out in cells with the volume of 500 mL; 50 mL of the respective TLC-systems were placed into them; the cell was saturated for 30 min. In 10 μg of efavirenz were applied on the start line in the distance of 1 cm from the plate edge. The solvents path length was 8 cm. After reaching the finish line by the mobile phase the plate was taken out from the cell, dried at the ambient temperature and developed with the respective reagents.

Results and discussion. It has been shown that a number of studied reagents (hydrogen peroxide solution, Nessler reagent, perchloric acid**,** FPN reagent, hydrochloric acid vapour, 1% $H_8[Si(W_2O_7)_6]$ solution, 1% $H_7[P(Mo_2O_7)_6]$ solution, 1% $H_7[P(W_2O_7)_6]$ solution, 1% $H_8[Si(Mo_2O_7)_6]$ solution, formaldehyde/sulphuric acid, Forrest reagent, Dragendorff reagent, 5% ferric chloride solution, iodoplatinate solution) do not colour efavirenz and such widely used colour reagents as UV-light, Erdmann reagent, Froehde reagent, Liebermann reagent, sulphuric acid, Marquis reagent, Mandelin reagent, acidified iodoplatinate solution, iodine vapour can be used for efavirenz detection on chromatographic plates.

Efavirenz gives positive detection results with the reagents used in analysis of barbituric acid derivatives (mercuric chloride/diphenylcarbazone reagent and cobalt nitrate/ammonia vapour) and the most of reagents used for detection and identification of the substances of basic nature, including so-called "generally alkaloid reagents".

Positive results have been recorded when developing efavirenz according to the scheme of TLC-screening of the substances of basic nature. Developing the plates with acidified potassium permanganate solution leads to formation of brown spots. Application of acidified ninhydrin spray results in brown spots. Overspraying the plates with FPN reagent and Dragendorff reagent followed by acidified iodoplatinate solution does not change the previous results.

Developing efavirenz according to the scheme of TLC-screening of the substances of acid and neutral nature leads to positive results (yellow spots after heating) with Van Urk reagent. Overspraying the plates with 5% ferric chloride solution does not change the previous results.

Chromatographic mobility of efavirenz has been studied in 17 solvents systems; the systems are used as standard mobile phases according to recommendations of the International Association of Forensic Toxicologists for TLC-screening of organic compounds of acid, neutral and basic nature, and also in the general TLC-screening of organic substances in Ukrainian forensic toxicological laboratories.

Conclusions. The behaviour of efavirenz when developing on TLC-plates with two types of substrate (plastic and glass) and with/without luminophor with commonly used coloured reagents has been studied. The R_f values of efavirenz under chromatographing conditions in the standard solvent systems used for TLC-screening of organic compounds of acid, neutral and basic nature have been set.