Materials and methods. Two types of chromatographic plates with a fixed layer of silica gel such as plastic plates with UV-indicator and glass plates without UV-indicator were used as the thin layers. After application of each reagent the plates were examined in visible and UV-light at two wavelengths (254 and 365 nm), then heated for 10 minutes at 110°C and viewed in visible and UV-light one more time.

Results and discussion. More than 50 reagents recommended «Clarke's...» (2011), their modifications, and a number of reagents proposed by us according to the structure of the analyte were used to visualize the spots of efavirenz on chromatographic plates.

The most sensitive developers are:

- concentrated sulphuric acid – light yellow spot;
- the Marquis reagent – light yellow spot;
- the Froehde reagent – brown spot;
- the Mandelin reagent – light brown spot;
- formaldehyde followed by the Mandelin reagent – pink spot;
- the Liebermann reagent – bright yellow spot;
- diphenylcarbazone and $HgSO_4$ – light violet spot;
- ninhydrin – light brown spot;
- acidified iodoplatinate solution – brown spot.

Conclusions. The set of developing reagents for efavirenz analysis by the method of TLC has been offered.

THE CHOICE OF CONDITIONS FOR DETERMINATION OF AMLODIPINE BY METHOD OF DERIVATIVE SPECTROPHOTOMETRY, SUITABLE FOR CHEMICAL-TOXICOLOGICAL ANALYSIS

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Introduction. Amlodipine besylate belongs to a group of blockers of calcium channels, derivatives of 1,4-dihydropyridine, is used to treatment of arterial hypertension and vasospastic forms of angina pectoris. Amlodipine reduces peripheral and coronary resistance, improves coronary blood flow, reduces intracellular overload with calcium, suppresses platelet aggregation. According to the literature sources, amlodipine in case of overdose can provoke the development of breast cancer, cause ischemia of the optic nerve. Deadly poisoning with amlodipine may accompany drug overdoses or suicidal cases. Fatal doses for children and adults range from 0,9 to 4,1 mg / kg.

Studies of biological objects in the forensic toxicology departments of the forensic medical examination bureau are conducted in the absence of control experiments. The choice of the method of derivative spectrophotometry for the identification and quantification of drugs in the chemical-toxicological analysis makes it possible to eliminate the influence of the background of impurities and to obtain reliable and reproducible results.

Aim. The aim of this work is the choice of the optimal conditions for the identification and quantitative determination of amlodipine by the method derivative UV spectrophotometry, suitable for chemical-toxicological analysis.

Materials and method. To select the optimal conditions for the analysis by the method of derivative spectrophotometry, UV light absorption spectra of the investigated amlodipine solutions were measured with a spectrophotometer SF-46 in the wavelength range 200-350 nm in a cuvette with a layer thickness of 10 mm. As a solution of comparison was used the corresponding solvent.

The second derivative of the absorption spectra ($d^2A / d\lambda^2$) was calculated using the least-squares polynomial approximation. The calculation of the second derivative for wavelength λ_3, the optical density of amlodipine solutions was measured at wavelengths λ_1, λ_2, λ_3, λ_4, λ_5 with an interval of 4 nm. The obtained values were multiplied by the polynomial coefficients for the corresponding number of points. Identification of the test substance was carried out in the presence of maxima, minima and points of

intersection of the derivative with the axes of wavelengths, which are more clearly manifested in the second derivative of the spectrum in comparison with the initial spectra of light absorption in the UV region of the spectrum.

Results and discussion. Identification of amlodipine with a second derivative was carried out in a 0,1 M solution of acid chloride in methanol at the presence of maxima at λ - 278, 342 ± 2 nm, minima at λ - 250, 366 ± 2 nm, and the points of intersection of the derivative with axes of wavelengths λ - 260, 352, 376 ± 2 nm, which provided the opportunity to obtain reliable results.

For the quantitative determination of amlodipine by the method of derivative spectrophotometry, the optical density of standard and model solutions was measured at wavelengths of 352, 359, 366, 371 and 376 ± 2 nm, a comparison solution of 0,1 M solution of acid chloride in methanol. At the same time, the second derivative was determined at the same wavelengths of the UV spectrum of the standard solution of amlodipine in a 0,1 M solution of acid chloride at a concentration of 40,0 μg / ml and the amlodipine content was calculated in model solutions.

As a result of calculations of metrological characteristics it was established that the relative uncertainty of the average result did not exceed ± 1,68%; the limit values of the confidence interval of the average result were $\overline{X} \pm \Delta\bar{x}$ = 99,54 ± 1,67 %; the relative standard deviation of the average result did not exceed – 0,60%.

For the application of the method of derivative spectrophotometry in the analysis of biological objects, it is necessary that the spectral characteristics of substances and impurities be approximated by polynomials of different degrees. The degree of polynomial for the spectral curve of a substance was higher than the polynomial for the spectral curve of biogenic impurities.

As a result of the research, it was found that the use of orthogonal functions was optimal for the determination of the investigated substances in the background of the linear absorption of impurities or solvent. Thus, in the area of maximum absorption of toxic substances, a linear dependence of the light absorption of the background of impurities on the wavelength was observed.

Quantitative determination of amlodipine was carried out by isolation with water, acidified with acid oxalic, from model mixtures of liver tissue of animal origin.

Extractive purification of extracts was carried out with diethyl ether at pH 2,0, TLC-purification of extracts was performed in the system of mobile solvents - methanol-chloroform (9:1), $Rf_{amlodipine}$ = 0,51-0,53. Location reagents - UV light and Dragendorff's reagent as modified by Mounier. The concentration of amlodipine in solution (C, μg / ml) was determined by the spectrophotometry method in the UV region of the spectrum at λ_{max} = 365 ± 2 nm, the comparison solution - the extract from the control sample - using the calibration graph or the corresponding equations of the linear dependence of the optical density and its concentration - A = 0,0329 + 0,0225 C.

At the same time quantitative determination of amlodipine in extracts was carried out by the method of derivative spectrophotometry. It was established that light absorption of extracts from control and liver tissue was approximated by polynomials of different degrees, with the degree of polynomial for the spectral curve of amlodipine was higher than the polynomial for the spectral impurity curve.

The results of quantification of amlodipine in extracts from the liver tissue by spectrophotometry in the UV region of the spectrum (34,8 ± 2,08%) and derivative spectrophotometry (31,5 ± 2,34%) were similar in magnitude.

When comparing variances for the F-criterion for derivative spectrophotometry and spectrophotometry in the UV region of the spectrum, it has been established that both methods do not have significant differences, and therefore they are practically equivalent. In the absence of control samples and the influence of the background of impurities, the method of derivative spectrophotometry provides an opportunity to obtain reliable and objective results.

Conclusions. According to the results of research, the method of identification and quantitative determination of amlodipine with the use of derivative spectrophotometry, suitable for chemical-toxicological analysis, has been developed. The possibility of using the method of derivative spectrophotometry for correction of the influence of coextractive substances on the results of analysis of biological objects is shown.