Results and discussion. In the kinetic method, the initial rate of reaction, V_0 is measured in one of many conventional ways, by following either the production of product or the disappearance of the substrate, enzyme, inhibitor and activator. It should be emphasized the rate method is faster, because the rate can be measured initially without having to wait for the reaction to go to completion.

Enzyme method can be used for determination substrate. The most important advantages of enzymatic assay of substrate are specificity and the great sensitivity. Glucose, for example, is oxidized at the rate of a new present per minute, regardless of concentration. Thus, a 10^{-7} M solution can be analyzed as easily as 10^{-4} M solution.A complete review of enzymatic methods for the assay of carbohydrates, amino acid, organic acid, hydroxyl compounds, esters, aldehydes, quaternary ammonium compounds, organophosphates, carbamates and inorganic substance has been prepared by Guilbault and Bergmeyer. Common techniques such as spectrophotometry, measurements of change in pH and manometric measurements have been described for the assay of almost all enzymes. With the advent of new techniques, electrochemical, fluorimetric and chemiluminometric as well as the successful immobilization of several enzymes, many of the difficulties have now been resolved. Because of their simplicity and susceptibility to automation, photometric methods have been used extensively to follow enzyme activity. For determination of activators: the initial rate of the enzyme reaction used because it is proportional to the activator concentrations. Determination of inhibitors: it is a compound that causes a decreased in the rate of enzyme reaction, either by reacting with the enzyme to form an enzyme – inhibitor complex or by reacting with the enzyme – substrate intermediate to form a complex:

$$E + I \rightarrow EI$$
$$E + S \leftrightarrow ES \leftrightarrow P + E$$
$$\uparrow\downarrow I$$
$$E - S - I.$$

In general, the initial rate of an enzymes reaction will decrease with increasing inhibitor concentration, linearly at low inhibitor concentrations, and the will increasing inhibitor concentration, linearly at low inhibitor concentrations, and then will gradually approach zero. Analytical working curves for inhibitor assay are generally constructed by plotting percent – age inhibitor against concentration of inhibitor. The percentage inhibition is calculated as follows:

$$Inhibition\ (\%) = (Rate_{\ No\ ingibitor} - Rate_{\ ingibitor}) / Rate_{\ No\ inhibitor} \times 100.$$

Generally, a graph of percentage inhibition against concentration is a typical exponential type of curve with a linear range extending from 0 to 60 or 70% inhibitor that causes a 50% inhibition of enzymic activity is termed the I_{50}, and is a measure of the strength of an inhibitor.Organophosphate and carbamate pesticides, insecticides, toxic metal ions, quaternary ammonium compounds, have been studied using enzymatic methods. A CL system based on three simultaneous coupled enzymatic reactions involving Acetylcholinesterase, Cholinoxidase and Horseradish peroxidase with Luminol as the CL substrate has been employed to assess the potency of Acetylcholinesterase inhibitors. The analytical procedures, based on the measurement of the kinetics of the CL emission, was very rapid and suitable for the high-throughput screening of Acetylcholinesterase inhibitors.

Conclusions. Enzymes have potential utility in analytical chemistry, and may be successfully applied in the pharmaceutical analysis. The specificity of enzyme is very highly, can solve the problem of analysis of one substance in the presence of many similar compounds in complex medicines and the problem of trace amounts analysis of preservatives.

DEVELOPMENT OF VORTIOXETINE DETECTION METHOD USING TLC AND CHROMOGENIC REACTIONS FOR CHEMICAL-TOXICOLOGICAL ANALYSIS

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Introduction. Vortioxetine (1-[2-(2,4-Dimethylphenylsulphanyl)-phenyl]-piperazine hydrobromide) is an antidepressant agent of a new generation. Information about detection and identification methods of Vortioxetine developed for toxicological studies has not been found in the available literature.

Aim. To develop sensitive, specific, also simple and accessible methods for Vortioxetine detection suitable for the purposes of chemical-toxicological analysis.

Materials and methods. Chromatographic mobility of *the antidepressant* in thin sorbent layers was studied in 12 mobile phases including those recommended by The International Association of Forensic Toxicologists (TIAFT) for TLC drug screening using Merk chromatographic plate. Mandelin's, Marqui's, Froehde, Lieberman, Erdman reagents, concentrated sulphuric, nitric, hydrochloric, perchloric, phosphoric and acetic acids were used as chromogenic reagents.

Results and discussion. The mobile phases of toluene – acetone – ethanol –25 % ammonia (45:45:7.5:2.5) (R_f = 0.37) and chloroform – dioxane – acetone – 25 % ammonia (47.5:45:5:2.5) (R_f = 0.45) were the most suitable for Vortioxetine identification. UV light (λ_{254}) (violet fluorescence, sensitivity was of 1.0 μg in the sample) and Dragendorff reagent with Munier modification (orange spots on the yellow background, sensitivity was of 1.0 μg in the sample) were used for visualization. The products of Vortioxetine interaction with the range of chromogenic reagents were of selective colour relating the endogenous biological matrix components. They were nitrate acid (lemon-yellow colour, sensitivity was of 5.0 μg), Froehde reagent (yellow colour turned into green, and then into blue, sensitivity was of 3.0 μg), Mandelin's reagent (green colour turned into blue, and then into brown, sensitivity was of 2.0 μg). Vortioxetine did not form the coloured products with Marqui's and Erdman reagents, hydrochloric, phosphoric and acetic acids.

Conclusions. Two mobile phases and four selective chromogenic reagents suitable for Vortioxetine detection in toxicological screening have been established. According to the TIAFT recommendations about the reliable identification of toxic substances in TLC-screening the acceptable condition is to use at least 2-3 mobile phases and four reagents on the same chromatographic plate consequentially.

METHOD OF DETERMINATION PYRETHROIDS IN ANIMAL ORIGIN OF OBJECTS

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Introduction. Today, pyrethroids are one of the most common pesticide groups. These are relatively new pesticides that have high insecticidal activity with pronounced selectivity. The selectivity of their actions is much higher than in most other drugs.

Aim. The aim of the work was to determine the optimum conditions for determining the residual amounts of pyrethroids in products of animal origin by means of gas-liquid chromatography.

Materials and methods. In the development of the method for determining the residual quantities of pyrethroids, standard pesticide solutions were used. To identify bifentrine, a gas chromatograph with an electron capture detector was used.

Results and discussion. At the first stage of determining the residual quantities of pyrethroids, the main task was to achieve maximum removal of the pyrethroid from the matrix. Experimentally testing such extragrants and their mixtures as acetone, chloroform, benzene, hexane, ethyl alcohol found that the best extractant of different chemical composition of the matrices is acetone. But when using acetone to remove a pesticide from a matrix, organic compounds such as fat, lipids, carotenoids, and the like also pass through it. Therefore, at the second stage, the extract had to be released from these undesirable compounds. The best way was to freeze with the addition of distilled water to the acetone extract. The optimum freezing time is one hour. The remainder is separated by filtration through a cotton filter.

Re-extraction was performed twice to completely redistribute the drug to hexane, using 10 and 15 cm^3 of extractant. Chromatographic columns were used to completely purify the extract, and bifentrine was eluted with hexane. The purified extract was analyzed on a gas chromatograph. The detection limit (LOD) is 1.4 ng, the limit of determination according to this procedure is 0.02 mg/kg.

Conclusions. When developing the method for determining bifentrine, it was established that the optimal conditions for extracting pyrethroid from the matrix are acetone extraction, precipitation of co-extracts from a water-acetone solution by freezing for one hour, redistribution in hexane, and purification of the extract on a chromatographic column.