DEVELOPMENT OF DERIVATIVE TLC-PURIFICATION PROCEDURE FOR DETERMINATION OF DOXYLAMINE IN BIOLOGICAL FLUIDS

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Introduction. One of the vital problems in the process of developing the analytical procedures for application in chemical and toxicological analysis is ensuring the necessary degree of specificity in relation to the components of biological matrix. The special importance this question acquires in the context of analysis of the biological objects exposed to the processes of putrefaction, burning, storages under various conditions for a long term, multiple freezing/thawing, etc. The solution of this problem lies in the plane of developing the effective purification methods for extracts from biological material.

Aim. The aim of the paper is to develop the purification procedure for extracts from blood and urine containing doxylamine by means of the method of derivative TLC, and to estimate the possibilities of application of the offered purification method in relation to the biological liquids exposed to the putrefaction processes.

Materials and methods. Doxylamine of pharmacopoeial purity was used in the experiment.

The model and also blank-samples were analysed for each developed procedure; the blank-samples were prepared in the following way: 5 samples (20.00 ml) of the blood obtained from the different sources, 1.00 ml of distilled water were added into them.

Validation of the procedures of doxylamine quantitative determination was carried out with application of the normalized coordinates; the application range was 25 – 175%; the number of concentration levels was g = 7. The doxylamine concentration in urine corresponding to the point of 100% was 32 μg/ml.

Results and discussion. Doxylamine isolation from biological liquids has been carried out using amphiphylic solvent (acetonitrile) under the conditions of aqueous phase saturation by electrolyte (ammonium sulphate); this approach enjoys wide popularity in modern forensic and toxicological analysis.

Isolation has been carried out in the weak-acid medium (pH = 5) that results in decreasing of co-extraction processes of biological matrix components in a number of cases. It is necessary to note that application of amphiphylic solvents and saturated solution of ammonium sulfate allows to maintain the isolation efficiency of substances of base character in the weak-acid medium at the same level as in the alkaline medium – it is conditioned by shift of pH real value in alkaline side for mixtures of electrolytes saturated solutions with amphiphylic solvents.

Purification of the extracts from blood and urine containing doxylamine has been carried out using the method of derivative TLC; generally this method provides obtaining the derivate for the substance to be investigated by means of the certain chemical reaction, its elution in the certain mobile phase, visualisation by the certain developer and determination of the value of R_f.

The offered method of derivative TLC-purification consists of applying the extracts from biological liquids on the start line of chromatographic plate (at the same time the standard sample is applied on the plate), eluting the chromatographic plate sequentially with the application of two mobile phases and eluting doxylamine by 0.01 mole/l hydrochloric acid solution from the chromatographic plate area corresponding to the spot of standard sample. Besides, it is provided obtaining the quaternary N-chlorammonium base for doxylamine in the way of processing the sample to be investigated and the standard sample by the excess of sodium hypochlorite solution in the saturated sodium hydrocarbonate solution on the start line of chromatographic plate after eluting the plate in chloroform (mobile phase 1). It is suggested to use the mixture of hexane and diethyl ether (2:1) as a mobile phase 2, and to carry out developing the spot of standard sample by 1% *p*-aminodiethylaniline sulphate solution.

Quantitative determination of doxylamine in eluates was carried out using extraction-photometric, UV-spectrophotometric and HPLC-procedures.

Determination and estimation of specificity and recovery for the developed procedures has been carried out, and the results allow to state the acceptable values of the parameters for all variants of final analytical operations.

For the possibilities estimation of applying the offered method of derivative TLC-purification in relation to the biological liquids exposed to the putrefaction processes 4 series in 6 blank-samples of biological liquids were prepared and exposed to storing at 25 ºC for 1, 2, 3 and 4 weeks respectively. For each series 3 blank-samples were spiked by analyte after respective expiry date, and then the analysis of all series were carried out.

The results of specificity and recovery determination for the developed procedure of derivative TLC-purification of extracts containing doxylamine in relation to the components of biological matrix exposed to the putrefaction processes allow to state the acceptability for all bioanalytical procedures.

Conclusions. The method of derivative TLC-purification of extracts from blood and urine containing doxylamine has been developed; the method allows to increase the elution degree of doxylamine from the chromatographic plate and decrease the amount of co-extractive substances in the obtained eluates. Possibilities of application of the offered method of derivative TLC-purification in relation to the biological liquids exposed to the putrefaction processes has been confirmed.