DEVELOPMENT AND VALIDATION OF THE HPLC-PROCEDURES OF DOXYLAMINE DETERMINATION IN BLOOD IN THE VARIANT OF THE METHOD OF STANDARD

Nemr Nur Eddin Fatima, Trut S. M., Klimenko L. Yu., Kostina T. A.
The National University of Pharmacy, Kharkiv, Ukraine
info@anchem.nuph.edu.ua

The purpose of the paper is developing the set of HPLC-procedures of doxylamine quantitative determination in blood using different variants of sample preparation, carrying out validation of the offered methods for choosing the optimal procedure of sample preparation provided effective doxylamine isolation from blood and low content of co-extracted substances in the obtained extracts at the minimum value of the method uncertainty, and also estimating the possibility of the method of standard application for doxylamine HPLC-determination in blood.

The HPLC-method for doxylamine determination using the system of HPLC-analyzer «Милихром А-02» was developed previously – the retention time of doxylamine was 11.85 min. We have suggested to carry out doxylamine isolation from blood using amphiphylic solvents with subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte; this approach enjoys wide popularity in modern forensic and toxicological analysis. Such amphiphylic solvents as isopropanol, acetonitrile and methanol have been used in the experiment; ammonium sulphate has been applied as electrolyte for saturation of aqueous phase.

Isolation has been carried out in the alkaline (pH = 11) and weak-acid medium (pH = 5); carrying out isolation of analytes from biological objects in the weak-acid medium 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.

For choosing the optimal method of doxylamine determination in blood we have carried out validation of all developed procedures by such parameters as specificity, recovery, linearity, accuracy, repeatability and intermediate precision in the variant of the method of standard.

The methods validation has been carried out at the first stage using model solutions. The total results of this stage allow to point to the conclusion about acceptable linearity, accuracy and repeatability of the HPLC-procedure of doxylamine quantitative determination in the variant of the method of standard that

gives the possibility to recommend it to further application in forensic toxicology with the purpose of development of the methods of biological objects analysis for doxylamine quantification.

At the second stage the methods validation has been carried out using model samples.

For specificity investigation for the developed procedures as for the components of biological matrix we have determined the sum of peaks areas on the chromatograms of blank-samples within 11^{th} and 12^{th} minutes – $S^{\Sigma blank}_{t_R \pm 0,5 \min}$.

The maximum peak area for doxylamine is observed in the case of detection at the wave length of 210 nm, but at the same wave length the sum of peaks areas is maximum on the chromatograms of blank-samples. At the same time the less intensive doxylamine peak at λ = 260 nm (this wave length is the nearest to characteristic line of doxylamine in UV-range of spectrum) is accompanied by the absence of peaks with the retention time, which is coincident with (or near to) the doxylamine retention time, on the chromatograms of blank-samples for all variants of procedures of analyte isolation from blood that points to the conclusion about acceptable specificity of the developed methods as for the components of biological matrix when using 260 nm as a working wave length.

The absence of peaks with the retention time, which is coincident with (or near to) the doxylamine retention time, on the chromatograms of blank-solutions for all wave lengths used for detection in the described HPLC-system is the evidence of the correct choice of sample preparation procedure for all considered cases.

It is necessary to note that in all cases carrying out doxylamine isolation from blood at pH = 5 provides lower sum of peaks areas on the chromatograms of blank-samples than in the case of alkaline pH using; at the same time by the results of recovery study small decreasing of doxylamine isolation efficiency from blood – within 3 – 5% – is noted under these conditions. The procedures with acetonitrile application are characterized by the best extraction efficiency.

The reproducibility of recovery values satisfies the acceptability criteria for all variants of methods.

Taking into account the data about specificity of the developed procedures the investigations of linearity, accuracy and precision have been carried out only for the set of procedures with application of the weak-acid medium for doxylamine isolation from blood.

All examined methods are characterized by the acceptable parameters of linearity, accuracy and precision, but high efficiency of doxylamine extraction from blood and low value of the method uncertainty allow to consider the method with acetonitrile application in the weak-acid medium as optimal for sample preparation of blood to further HPLC-determination of doxylamine.