DEVELOPMENT AND VALIDATION OF THE METHODS OF CAPTOPRIL SPECTROPHOTOMETRIC DETERMINATION IN BLOOD BY THE REACTION WITH THE ELLMAN REAGENT

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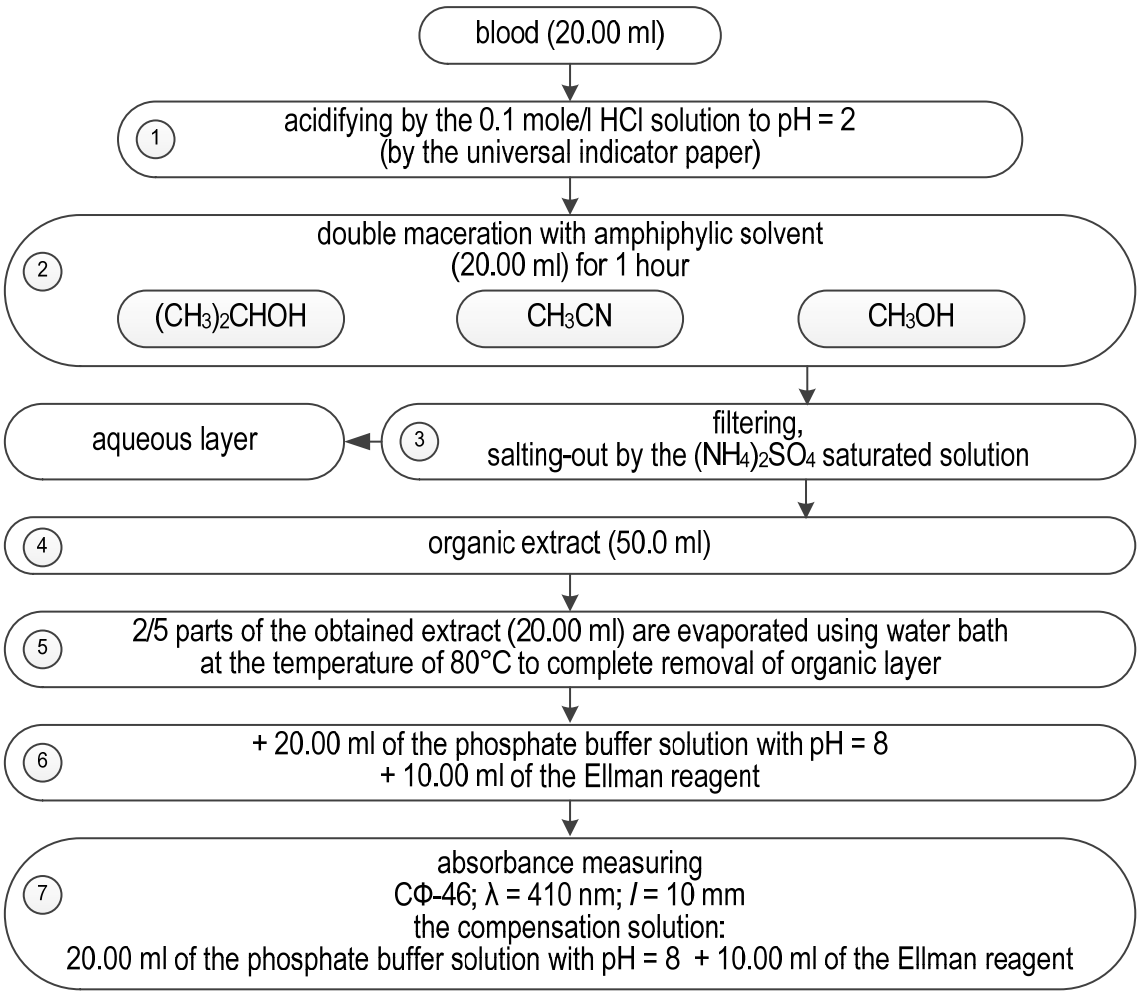
The purpose of our paper is developing the set of methods of captopril quantitative determination in blood using different procedures of sample preparation based on spectrophotometric method by the reaction with the Ellman reagent, carrying out validation of the offered methods and choosing the optimal procedure of sample preparation provided effective captopril isolation from blood and low content of co-extracted substances in the obtained extracts at the minimum value of the method uncertainty.

The design of experiment on development of methods is presented on the scheme.



For captopril determination the spectrophotometric method based on the photometric reaction with the Ellman reagent at pH = 8 has been developed by us.

In the present paper it has been suggested to carry out captopril isolation from

blood by its maceration with amphiphylic solvents and subsequent separation of organic layer under the conditions of aqueous phase saturation by electrolyte for increasing the efficiency. Such amphiphylic solvents as methanol, isopropanol and acetonitrile were used in the experiment; ammonium sulphate was applied as electrolyte for saturation of aqueous phase; isolation was carried out in the acid medium (pH = 2).

Thus, the development of the set of methods of captopril determination in blood using the method of spectrophotometry by the reaction with the Ellman reagent has been become the result of this stage of investigations; the methods differ by the procedures of sample preparation.

For choosing the optimal methods of captopril determination in blood we carried out their validation by such parameters as specificity, recovery, linearity, accuracy, repeatability and intermediate precision.

The methods validation was carried out at the first stage using model solutions – the results of validation allow to point to the conclusion about acceptable linearity, accuracy and repeatability of the method of captopril quantitative determination by the method of spectrophotometry by the reaction with the Ellman reagent.

The results of specificity study show that carrying out captopril isolation from blood using amphiphylic solvents provides low contribution of biological matrix components into the absorbance of the sample to be analysed. It is possible to point to the conclusion about high efficiency of captopril isolation from blood – not less than 90% – by the results of recovery study. The method with acetonitrile application is characterized by the best extraction efficiency.

The values of reproducibility for recovery and blank-samples absorbance satisfy the acceptability criteria for all variants of the methods. The absorbance values obtained for the blank-solutions are the evidence of the correct choice of sample preparation procedure for all considered cases.

On the whole, all examined methods are characterized by the acceptable parameters of linearity, accuracy and precision, and the obtained data are the evidence of application possibility of the developed methods for captopril spectrophotometric determination in blood by the reaction with the Ellman reagent.

Thus, we have developed the set of spectrophotometric methods of captopril quantitative determination in blood by the reaction with the Ellman reagent using amphiphylic solvents (isopropanol, acetonitrile, methanol) for analyte isolation from matrix under the conditions of aqueous phase saturation by ammonium sulphate. Acetonitrile application in the acid medium (pH = 2) is optimal – contribution of matrix components into the absorbance of the sample to be analysed does not exceed 10%, extraction efficiency is ~97%.