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ANALYSIS OF DRUGS IN PHARMACOKINETIC STUDIES

Kurbonova Nargiza Ulmasovna

Assistant of the Department of Pharmaceutical Sciences

Andijan State Medical Institute

Abstract: A review of sensitive and specific analytical methods used in studying the pharmacokinetics of drugs is provided. The advantages and limitations of using enzyme immunoassay, a method of high-performance liquid chromatography with fluorescence and mass spectrometric detection, are shown. The use of one or another method in assessing the pharmacokinetics of drugs in each specific case is determined by the structure of the compound being studied and the equipment of the laboratory.

Keywords: Fluorescence and mass spectrometric detection, liquid chromatography, method, enzyme immunoassay, pharmacokinetics.

INTRODUCTION

The study of pharmacokinetics is based mainly on assessing the concentration of a drug substance (drug) in the patient's body at certain points in time after taking the drug. The objects of research are blood (whole, serum, plasma), urine, saliva, feces, bile, amniotic fluid, etc. The most accessible and most often tested are blood and urine samples.

Measuring the concentration of a drug can be divided into two stages: 1 - isolating a specific drug substance from a biological object, concentrating the test compound, separating it from the main endogenous components; 2 - separation of a mixture of compounds, identification of drugs and quantitative analysis.

MATERIALS AND METHODS

Studying the concentration of a drug in the blood provides information about the duration of circulation of the drug in the body, the bioavailability of the drug, the effect of concentration on the pharmacological effect, therapeutic and lethal doses, and the dynamics of the formation of active or toxic metabolites.

Studying the drug concentration in urine allows us to assess the rate of drug elimination and renal function. The concentration of metabolites in urine is an indirect indicator of the activity of metabolizing enzymes.

The study of biological material includes measuring the mass (volume) of the sample, releasing the drug (metabolites) from the sample cells, separating whole cells (for example, in the analysis of blood) or parts of cells (in the analysis of tissue homogenates), adding an internal standard, separation of proteins, sample purification (centrifugation, filtration), procedures for extraction, re-extraction, concentration and transformation of test substances into derivatives convenient for analysis, basic procedures for processing blood and urine samples, respectively.

RESULTS AND DISCUSSION

An ideal analytical method for measuring drug concentration should have high sensitivity, specificity and reproducibility, the ability to work with small volumes, ease of material preparation, low cost and ease of equipment maintenance, reliability and the possibility of

automation, ease of staff operation and versatility (the ability analysis of various classes of drugs).

To obtain reliable data, it is necessary to make an adjustment for the stability of the active substance and/or product(s), as well as the degree of its biotransformation in the analyzed biological media [1].

Validation of a method should be based on its intended application, and calibration should take into account the concentration range of the test sample. It is strongly discouraged to use two or more methods for analyzing samples on the same material with similar calibration ranges.

High performance liquid chromatography with fluorescence detection

In HPLC, the detector generates an electrical signal whose strength is proportional to the concentration of the analyte dissolved in the mobile phase. In the first liquid chromatographs (ion exchange), the mobile phase passing through the column with the sample components was collected in small vessels, and then using titrometry, colorimetry, polarography, etc. the content of the component in this portion was determined. In other words, the processes of separating a sample and determining its quantitative composition were separated in time and space. In a modern liquid chromatograph, these processes are provided by one device.

To detect sample components, any physical and chemical property of the mobile phase (absorption or emission of light, electrical conductivity, refractive index, etc.) can be used, which changes in the presence of molecules of the compounds to be separated. Of the 50 existing physicochemical detection methods, 5–6 are currently actively used.

Sensitivity is the most important characteristic of a detector. If sensitivity is determined through the double amplitude of the noise of the zero line, and the noise is expressed in physical units, then the sensitivity of the photometric detector will be expressed in units of optical density, refractometric - in units of refractive index, voltammetric - in amperes, conductivity detector - in units of refractive index. ric - in Siemens. In pharmaceutical analysis, sensitivity is expressed in terms of the minimum amount of analyte [2].

Despite the fact that currently 80% of chromatographs are equipped with spectrophotometric detectors as standard, fluorescence detection is becoming increasingly widespread, especially when determining the concentration of compounds that can “glow” under the influence of exciting radiation. The luminescence intensity is proportional to the intensity of the exciting light. The study of emission spectra (fluorescence and phosphorescence) is a more sensitive and specific method than the study of absorption spectra.

CONCLUSION

When choosing an analytical method, it is necessary to keep in mind that the use of ELISA is limited by the availability of required reagents, fluorescent detection, and the need for the test compound’s own fluorescence. Although the above limitations are not significant for mass spectrometric detection, the cost of equipment today remains quite high, and this type of analysis requires special skills.

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