



MODERN ANALYTICAL METHODS FOR THE QUANTITATIVE DETERMINATION OF ENZYMES AND THEIR APPLICATION IN PHARMACEUTICAL ANALYSIS

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Relevance. Enzymes are high-molecular-weight biological catalysts that facilitate biochemical reactions that occur in living organisms and involved in virtually all stages of metabolism and energy exchange. Their catalytic activity plays a crucial role in regulating cellular metabolism, coordinating physiological processes, and maintaining homeostasis. Alterations in enzyme activity can lead to the development of numerous diseases; therefore, their quantitative determination is considered an important area of clinical diagnostics, biotechnology, and pharmaceutical analysis [1,2]. In the pharmaceutical field, quantitative determination of enzymes plays an important role in the production of biological and biotechnological preparations, quality control of enzyme-containing medicinal products, conducting bioequivalence studies, and evaluating the pharmacokinetic properties of biologically active substances. The development of modern analytical technologies, such as spectrophotometry, fluorimetry, enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (LC–MS/MS), and biosensor technologies, has significantly improved the sensitivity and accuracy of methods



used for enzyme quantification [3,5]. These methods enable reliable assessment of the biological activity of enzymes, quality control of pharmaceutical products, and improvement of the diagnostic accuracy of clinical laboratory analyses.

Aim of the study. To analyze modern analytical methods for the quantitative determination of enzymes based on the scientific literature, to comparatively evaluate their principles of operation, analytical capabilities, advantages, and limitations, and to identify the prospects for their application in pharmaceutical analysis and clinical diagnostics.

Materials and methods. The study was based on a literature review of scientific sources related to methods for the quantitative determination of enzymes were examined. Articles indexed in the PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar databases were used for the analysis, along with international textbooks and monographs on enzymology, biochemistry, and pharmaceutical analysis. The sources were selected based on their scientific reliability, relevance, and relevance to the research topic. The obtained data were summarized using comparative, systematic, and analytical approaches, and the analytical performance characteristics of classical and modern analytical methods for the quantitative determination of enzymes, as well as their significance in pharmaceutical practice, were evaluated.

Discussion. The literature review showed that the main parameter for the quantitative determination of enzymes is their catalytic activity. Since absolutely specific chemical reagents for enzymes are not available, their amount is often assessed not directly, but rather through the rate of the reaction they catalyze. Therefore, enzyme activity is evaluated based on the determining the decrease in substrate concentration or the rate of formation of the reaction product over a specific time interval. This approach constitutes the methodological basis of enzymology and pharmaceutical analysis [1,2]. Classical methods for determining enzyme activity are based on recording changes in the physicochemical properties of the substrate or reaction product. In particular, changes in optical density, viscosity, electrical



conductivity, refractive index, and optical rotation characterize the progress of an enzymatic reaction. For example, the activity of the enzyme amylase is determined based on the amount of reducing sugars formed as a result of starch hydrolysis, whereas the activity of pepsin is determined based on the degree of degradation of protein substrates. Although these methods are simple and cost-effective, their sensitivity and selectivity are limited compared with modern instrumental methods [2,6].

At present, several modern methods are used for the quantitative determination of enzymes. Among these methods, spectrophotometry is one of the most widely used. This method is based on measuring changes in the absorbance of light at a specific wavelength by the substrate or reaction product during an enzymatic reaction. The rapidity of spectrophotometric analysis and its relatively simple procedure make it widely applicable in clinical biochemistry laboratories and for quality control in pharmaceutical manufacturing facilities [3,7].

Among immunochemical methods, **enzyme-linked immunosorbent assay (ELISA)** is distinguished from other methods by its high sensitivity and selectivity. This method is based on an antigen–antibody reaction and enables the detection of enzymes and enzyme-related biomarkers at very low concentrations in biological fluids. For this reason, ELISA is widely used in clinical diagnostics, pharmaceutical research, and quality control of biological preparations [4,8].

High-performance liquid chromatography (HPLC) provides high accuracy and reproducibility in the separation and quantitative determination of enzymatic reaction products. This method offers high selectivity in complex biological samples and is widely used to study enzymatic processes, evaluate the effects of medicinal products, and analyze biologically active substances. The main advantages of HPLC include high accuracy, good separation efficiency, and reliable results. However, the high cost of the equipment, relatively long analysis time, and the need for qualified personnel are considered its main limitations [5,8].



Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is considered one of the most sensitive and selective methods in modern bioanalytical research. This method enables the detection of enzymatic reaction products at very low concentrations, simultaneous analysis of multiple analytes, and highly accurate quantitative determination of enzymes in complex biological matrices. Therefore, LC–MS/MS is currently widely used in pharmacokinetic studies, standardization of biological preparations, and biomarker determination [5,9].

In recent years, **biosensor technologies** have been developing as one of the promising approaches for the quantitative determination of enzymes. Electrochemical, optical, and piezoelectric biosensors enable real-time monitoring of enzymatic reactions, reduction of analysis time, and obtaining highly sensitive results even from small volumes of biological samples. In addition, their portability and integration into automated analytical systems have made biosensors particularly important in modern laboratory diagnostics. Nevertheless, their practical application is associated with certain limitations related to sensor stability, as well as calibration and validation processes [9,10].

Comparative analysis of various analytical methods has shown that each method has its own advantages and limitations. For example, while the spectrophotometric method is simple, rapid, and economically advantageous, the ELISA method provides high selectivity and sensitivity; HPLC and LC–MS/MS methods enable quantitative analysis of complex biological samples with high accuracy, whereas biosensors are distinguished by their ability to provide real-time monitoring. Therefore, in pharmaceutical analysis, the selection of an analytical method should be based on the purpose of the analysis, the characteristics of the biological sample, the required level of accuracy, and the technical capabilities of the laboratory [3,10]. The selection of methods for the quantitative determination of enzymes depends on their sensitivity, selectivity, accuracy, analysis time, and cost-effectiveness. Currently, instrumental methods that provide high analytical performance are gaining priority in modern pharmaceutical analysis. Therefore, in



this thesis, the analytical methods most widely used in practice were comparatively analyzed.

Conclusion. The literature review conducted demonstrated that **assessment of catalytic activity** is the principal analytical criterion for the quantitative determination of enzymes. Modern analytical methods, including spectrophotometry, ELISA, HPLC, LC–MS/MS, and biosensor technologies, enable the determination of enzymes with high accuracy, sensitivity, and selectivity. Each method has its own advantages and limitations, and the selection of an appropriate method for a particular analysis depends on the purpose of the analysis, the characteristics of the biological sample, and the required analytical performance characteristics. The application of these methods in pharmaceutical analysis is of great importance for quality control of biological preparations, standardization of enzyme-containing preparations, and improving the reliability of clinical diagnostics. Further advancement of modern analytical technologies is considered one of the promising directions for the development of enzymology and pharmaceutical analysis.

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