UDC 616-097:57.083,571.27

INNOVATIVE IMMUNOLOGICAL METHODS FOR QUALITATIVE DETERMINATION OF LOW-MOLECULAR SUBSTANCES IN BIOLOGICAL OBJECTS

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✓ ***Resume,***

The article presents materials on the study of methodological methods for the practical use of enzyme immunoassay in order to determine low-molecular happens in biological objects by immunoextraction of determined compounds using immobilized antibodies (sorbents). It is shown that for effective quality detection of these substances, it is necessary to make the right choice of carrier and enzyme label.

Keywords. Enzyme-linked immunoassay (ELISA), enzyme label, narcotic drugs, psychotropic substances, chemical modifications, preliminary tests.

ИННОВАЦИОННЫЕ ИММУНОЛОГИЧЕСКИЕ МЕТОДЫ КАЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ НИЗКОМОЛЕКУЛЯРНЫХ ВЕЩЕСТВ В БИОЛОГИЧЕСКИЕ ОБЪЕКТЫ

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✓ ***Резюме,***

В статье представлены материалы по изучению методических методов практического использования иммуноферментного анализа для определения низкомолекулярных явлений в биологических объектах путем иммуноэкстракции определенных соединений с использованием иммобилизованных антител (сорбентов). Показано, что для эффективного определения качества этих веществ необходимо сделать правильный выбор носителя и метки фермента.

Ключевые слова. Иммуноферментный иммуноанализ (ИФА), ферментная метка, наркотические средства, психотропные вещества, химические модификации, предварительные тесты.

BIOLOGIYA OBEKTLARIDA PAST MOLEKULYAR BIOLOGIK MUHITNI SIFATINI INNOVATSIJN IMMUNOLOGIK USULLARDA ANIQLASH

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✓ ***Rezyume,***

Maqolada immunitetni pasaytiruvchi antitelalar (sorbentlar) yordamida ba'zi birikmalarni immunoekstratsiya qilish orqali biologik ob'ektlarda past molekulyar og'irlikdagi hodisalarni aniqlash uchun ferment bilan bog'liq immunosorbent tahlilini amaliy qo'llash bo'yicha uslubiy usullarni o'rganish bo'yicha materiallar keltirilgan. Ushbu moddalarning sifatini samarali aniqlash uchun fermentning tashuvchisi va etiketkasini to'g'ri tanlash zarurligi ko'rsatilgan.

Kalit so'zlar: Immunoassay fermenti (ELISA), ferment yorlig'i, dorilar, psixotrop moddalar, kimyoviy modifikatsiyalar, dastlabki sinovlar.

Relevance

One of the most important problems in the fight against illicit trafficking in narcotic drugs and psychotropic substances is the non-medical use of new psychoactive substances, the legal status of which has not yet been determined. This creates difficulties in establishing control measures for them, as well as conditions for illegal trafficking of such substances across the state border of Uzbekistan. Today, as a result of an experimental search based on chemical reaction between certain chemicals, designer drugs are created that differ in molecular composition from known narcotic drugs and psychotropic substances. These substances are called designer drugs and belong to the group of synthetic cannabinoids "spice". Given the rapid appearance of their modifications, the problem of developing simple, expressive, sensitive methods of determining them using the enzyme immunoassay (ELISA) becomes urgent.

Currently, detection and seizure of illicit traffic narcotic drugs, psychotropic substances requires the use of special technical developments to ensure objectivity, selectivity, fidelity active component (substance), performance, absence of false results and therefore

completeness of implementation of measures of restraint by law enforcement authorities against persons engaged in this kind of business. Solution of issues related to counteraction to spreading of drug addiction, causes the development of accessible and reliable methods for identifying facts of abuse of drugs and their illegal movements across the customs border of the state. In this regard, the development and production of test - systems for reliable diagnosis of abuse and violation of various kinds of drugs is one of the urgent tasks in the field of biotechnology. Given the above, the objective of this work was to systematization and generalization of the results of studies on the selection of the necessary conditions for enzyme immunoassay drugs and other intoxicating substances. It should be noted that in modern practice law enforcement particular practical application is found with immunoenzyme analysis methods, built on the interaction of antigen and antibodies and used to define a variety of physiologically active substances and objects - from low-molecular regulators of metabolism markers to cancer cells and pathogens. Thus, as diagnostic agents may be used hydrosols: ultrafine particles of metal oxides side subgroups and complex compounds on the surface of which certain technology dealt antigens or antibodies. It should be noted that the quantitative study of physico-chemical regularities of the reactions of antigens of different nature, are very important for the understanding of the immune interactions in vivo and to create immunochemical systems for detection of biologically active compounds. Theoretically EIA is based on the data of modern immunochemistry and chemical enzymology, knowledge of physical and chemical regularities of the reactions of antigen - antibody, as well as on the main principles of analytical chemistry. The sensitivity of the ELISA and time of the meeting is determined by several key factors: kinetic, thermodynamic properties of reaction antigen - antibody, the ratio of reagents, enzyme activity and resolution of the methods of its detection. Literary sources testify to the fact that any case of ELISA includes three stages, in particular:

4. Stage of recognition of the tested compounds specific to him antibody that leads to the formation of immune complex;

5. The formative stage of the connection conjugates with an immune complex, or with free places binding;

6. Stage of transformation of the enzyme label to the registered signal.

Currently it is known that in the modern practice of forensic medicine, pharmaceutical, toxicological analysis, including to determine narcotic and toxic substances has developed effective methods for determination of enzyme-linked immunosorbent assay amphetamine and antibodies to it in the biological liquids of the person, and also optimized the conditions detection of antibodies to amphetamine in human serum-based use immunoenzyme analysis of new synthetic antigens [1].

In immunoenzymatic methods for detection reaction "antigen - antibody" as a label (marker) are enzymes that represent various oxidase-oxidizing bacteria (special chemical substance - addition of a chromogen substrate) with the formation of colored products, intensity of colouring which is judged on the presence or absence of narcotic drugs in the analyzed sample. Technique of execution there are two types of immune-enzyme analysis: homogeneous and heterogeneous.

In the homogeneous EI.1SA all components of reaction - antibodies (antiserum), determined b> the substance, labeled determined by the substance (conjugate), the addition of a chromogen substrate are one aggregate state - solution. As marks are used enzymes such as lysozyme, glucose - 6 - phosphate dehydrogenase, malate dehydrogenase. When you link labeled gapten with antibody activity of an enzyme - label in the reaction with chromogene substrate is reduced through spatial changes of the protein enzyme, i.e. blocked access substrate to a fermentative centers and comes braking. In this case colouring of the analyzed solution after addition of the substrate will be directly proportional to the concentration of which has not entered into binding of the antibody labeled gapten, i.e. directly proportional to the concentration of rival drug, which is in the study of biological liquids.

In heterogeneous El ISA provides for phase separation of reactants - addition of a chromogen substrate is added after the stage of washing. Holding of incubation of the reaction mixture and linking gapten (labeled and unlabeled) antibodies on the solid basis ends removed from the reaction mixture unreacted reagents. The added addition of a chromogen substrate interacts with the enzyme - labeled associated with antibody immobilized on the solid medium with education in a particular coloring. If the concentration of the designated substance in the sample is significantly higher than the concentration gapten, labeled enzyme, after the removal of the last of the reaction mixture as a result of cleaning, the addition of a chromogen substrate will not form color (positive result). As the label in a heterogeneous method uses such enzymes as peroxidase, glycosidase, alcolin - phosphatase, at least - acetyl cholinesterase, glucoamylase and glucose oxidase [6].

Today, enzyme-linked immunosorbent assay is one of the perspective directions of immunochemical determination of biologically active substances (hormones, enzymes, antibiotics, drugs, combining the specificity of the immunological recognition and very high sensitivity detection enzyme labels, which can be applied most active enzymes. The discovery of the possibility of immobilization of the antigen and the antibody to the various media with preservation of their binding activity has allowed expanding the use in various fields of biology and medicine. Thus, as a solid phase, you can apply different materials, in particular, polystyrene, polyvinyl chloride, polypropylene and other high-molecular compounds.

In the work it was noted that a correct description of the interaction of antibodies with polyvalent antigens, which include oligomeric proteins and synthetic conjugates "gapten - media" (the drug), is a rather complicated problem due to a lot of the staging of a process and education of a large number of complexes of different composition. As artificial polyvalent antigens - liposomes allow properly characterize the influence of physico - chemical properties and composition of the antigens on the kinetic and equilibrium parameters of immunochemical reactions [21.

Astashkina O.G. developed the method of enzyme immunoassay for the detection of drugs group of opiates [3]. Research results, confirmed by standard chemical methods (thin-layer chromatography, gas chromatography-mass- spectrometry, high-performance chromatography) show that the method of enzyme immunoassay using diagnostic has high sensitivity and specificity, and can be recommended in forensic and customs practice to establish a group of drugs opiates in biological liquids in examination.

Recent advances in molecular biology, molecular genetics, bioorganic chemistry, and their practical implementation is allowed to authors of works to conduct studies of the influence of the properties immune reagents on kinetic and equilibrium parameters of the reaction "antigen - antibody". On the basis of the data developed models

of processes of formation of immune complexes are studied structural changes of protein antigens in the interaction with the antibodies, in particular, the modulation of the catalytic activity of enzymes antibodies. As model antigens in the study of immunochemical processes have been used hormones, enzymes, antibiotics, drugs. For the detection of these compounds proposed new formats analysis and marker system, designed to improve the sensitivity and expressiveness the definition [4].

As already noted, immunochemical methods for detection of narcotic substances differ necessary sensitivity and high specificity, resistance to interference factors of biological liquids. Currently known immunochemical methods of determination of different classes of drugs are based on the application- labeled analytes, capable of defining the necessary object or specific antibodies.

Biotechnology industry, the USA, Great Britain and France to produce the corresponding sets of reagents for radio prepare, immunofluorescence and enzyme immunoassay various [5]. It should be noted that the labeled antigen (AG*), when added intoxicants in the analyzed sample of known concentration, will combine with the antibody to [AG*-At] form complex.

It should be noted that currently developed, immunochromatographic analysis methods, aimed at the determination of the existence of certain concentrations of substances in biological materials, and is based on immersion test in physiological fluid that starts migrate along a strip on the principle of thin- layer chromatography, i.e. the formation of immune complexes between antibody immobilized on the chromatographic paper, and present in the analyzed sample free drug proportional to its concentration. Then strip is placed in a solution containing: a) conjugate (a certain drug - peroxidase); b) glucose; c) addition of a chromogen substrate (chlorine naphthol). During incubation conjugate binds with vacant active center of antibodies and interaction with the immobilized glucose oxidase, there is a consecutive generation of hydrogen peroxide, which contributes to the substrate oxidation with the formation of insoluble colored product. The concentration of antigen (defined substances) in the studied sample is inversely proportional to the intensity developed on a strip of color, i.e. the concentration of a substance is determined not by the enzymatic activity of the conjugate, and chromatographic behavior of.

Thus, being an effective method of diagnosis, rapid tests allow you to visually within a few minutes to determine and assess the content of the antigens, antibodies, hormones and drugs in biological fluids. The essential hematocrit, i.e. the ratio of plasma and formed elements. At high hematocrit reduces the number of plasma with an antigen, migrating along a strip. Given the above, of particular interest, from a practical point of view, is the use of marked imminoxyl radical gaptens in determining the concentration of morphine in biological liquids. For the drug antibodies were obtained and used in the test system after joining imminoxyl radical. If in the sample contained detectable drug, then he blocked the active centers of antibodies. Added as component test system morphine, labeled imminoxyl radical remained unbound and range of labels coincides with the spectrum of imminoxyl in the solution [6].

In the work developed effective immunological method of determination of opiates, cannabinoids, barbiturates in urine based on the reaction latex agglutination with the use of functional polystyrene and polymethylmethacrylate microspheres. Special practical interest is the development of the ELISA method of analysis of amphetamine and getting biochemical reagents (antibodies and antigens), because their qualitative characteristics are the main factors of analysis, constituting the foundation of the biotechnology development [1,8].

Studied solid-phase FI ISA method of determination of amphetamine included immobilization antigen conjugated on polystyrene tablet; competitive binding of free derivative of amphetamine, present in the test specimen and adsorbed on solid phase conjugate amphetamine protein with specific antibodies; identification of the resulting immune complex with anti-species antibody labeled with horseradish peroxidase; measurement of enzymatic activity in the resulting immune complex. Synthesized sorbents for basis of the conducted researches in the work affine chromatography, conducted selection of antibodies to amphetamine of biological liquids, optimized conditions of the analysis of immunoglobulin M and A in biological fluids based on synthetic antigens [1,9].

Given the foregoing, high sensitivity, combined with the rapidity of analysis, the possibility of simultaneous testing of a large number of samples and the lack of special need of preliminary clearance operations and concentration of the analyzed compounds in the sample, give ELISA advantages over other analytical methods and can be used for screening diagnostics. In this regard, today IFA is widely used not only in health care, various sectors such as agriculture, industrial biotechnology, but in the customs, forensic medical, pharmaceutical and toxicological practice with the purpose of revealing of drugs, counterfeit drugs, and a variety of illegally sold their mixtures and modifications.

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Entered 10.03.2020