Research on Biopharmaceutical Equipment and Separation and Purification Technology Based on Antibody Drugs

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Keywords: Antibodies; Biopharmaceutical equipment; Separation and purification technology

Abstract: The broad market prospect of antibody drugs makes the research and development of such drugs gradually enter the peak period. It involves a wide range of technical fields, high research and development costs and a long period of time, which are also incomparable to general genetically engineered drugs. Conventional antibody separation and purification methods include salting out method, ultrafiltration method, chromatographic separation method, etc. At the same time, the study of antibody diversity and epitope groups is also an important way to reveal the immune mechanism of life. However, biopharmaceutical equipment is a very critical presence in the biopharmaceutical industry, so relevant equipment must be used for operation in order to carry out biopharmaceutical. Therefore, strengthening the maintenance and management of modern biopharmaceutical equipment can effectively promote the production benefits of modern biopharmaceutical enterprises and ensure the maximization of their economic benefits. In this paper, the biopharmaceutical equipment and separation and purification technology based on antibody drugs are studied.

1. Introduction

Antibodies have the characteristics of high specificity and affinity for corresponding antigens, and have low toxic and side effects, thus having obvious specificity advantages in the diagnosis and treatment of diseases. In recent years, with the development of immunology and molecular biology technology and the elucidation of antibody gene structure, DNA recombination technology has begun to be used for antibody modification. There are about 500 kinds of diagnostic and therapeutic antibodies, and more than 100 kinds of antibodies are currently undergoing clinical trials [1]; At the same time, the study of antibody diversity and epitope groups is also an important way to reveal the immune mechanism of life. Among them, the scale of biopharmaceutical industry is increasing, and its main purpose is to serve biopharmaceuticals. This is not only reflected in the renewal of relevant equipment, but also in the improvement of relevant technologies, which does not provide better help for Chinese pharmaceutical enterprises to produce high-quality drugs, nor does it lay the foundation for the progress of the whole industry [2]. Many pharmaceutical companies in China are trying their best to catch up with the progress and enter clinical trials quickly, while ignoring the development of early mass production technology, resulting in product quality problems in the formal production stage. Common separation and purification methods include chromatography and non-chromatography. Generally, non-layer method can effectively reduce the content of DNA, viruses, miscellaneous proteins and other substances in the raw material liquid, and then chromatography is used for further purification. In this paper, the biopharmaceutical equipment and separation and purification technology based on antibody drugs are studied.

2. The Importance of Antibody Drugs and Separation and Purification Technology for Biopharmacy

The broad market prospect of antibody drugs makes the research and development of such drugs gradually enter the peak period. It involves a wide range of technical fields, high research and development costs, and a long period of time, which are incomparable to general genetic engineering drugs. The single dose of antibody drug is large, and the quality standard of the drug is

  DOI: 10.25236/isbsbh.2020.007

high. In particular, the characteristics of limited targets of antibody drugs and the effectiveness of their clinical application have led many research institutes and pharmaceutical companies in China to join the ranks of antibody biological development. In addition to achieving the goal of clarification, large-scale production and purification should also remove some host proteins or host DNA impurities as much as possible. The antibody is produced and screened in vivo or in vitro. Completely different from the human environment, the selected antibody does not necessarily have the best therapeutic effect and requires a lot of manpower, material resources and time. Human's understanding of life is transforming into concrete social benefits. The introduction of generations of bio-drugs not only brings new life light to patients, but also creates rich returns for investors. According to the therapeutic aim and target, the corresponding antibody size, the number of antibody binding values and the form of fusion protein are designed on the premise of keeping antigenicity and affinity unaffected.

It is generally believed that after a series of genetic engineering antibody modifications such as chimeric antibody, CDR transplantation and surface remodeling, along with the improvement of humanization degree, antibody immunogenicity decreases in turn [3]. The fine purification method for large-scale antibody drug production is mainly a three-step method [4], as shown in Table 1.

Table 1 Fine purification method for large-scale antibody drug production

Initial capture	Intermediate purification	Final refining
Protein a affinity chromatography	Anion exchange chromatography	Cation exchange chromatography
Protein a affinity chromatography	Mixed mode chromatography	Anion exchange chromatography
Protein a affinity chromatography	Hydrophobic interaction chromatography	Anion exchange chromatography

Conventional antibody separation and purification methods include salting out method, ultrafiltration method, chromatographic separation method, etc. In the specific application, the separation and purification effect is realized by filtering the polymer compound and solute molecules in the liquid mixture. The precipitation method is simple to operate, the separation efficiency is not high, and the high concentration salt solution is easy to inactivate the antibody, so the operating conditions should be controlled during the operation. Only by ensuring that the extraction technology meets the relevant requirements, can we ensure that the extraction has a more ideal effect, and ensure the quality of drugs and specific therapeutic effects. Therefore, the technical level of separation and purification directly affects the core competitiveness and market economic benefits of pharmaceutical enterprises [5]. Whether it is a tube centrifuge or a dish centrifuge, the feed rate and slag discharge frequency are the two key parameters that affect the clarification effect of the feed liquid, which need to be optimized to achieve the purpose of clarification. The screening of antibody target molecules, the preparation of human antibodies and all-human antibody libraries, and the large-scale high-throughput screening and optimization of antibody drugs are the main challenges in antibody drug research. Most antibodies (such as chimeric, humanized or bivalent antibodies) need to be expressed in mammalian cells. There are relatively few efficient expression vectors with independent intellectual property rights, and the expression level is not high enough. Therefore, some relatively economical and efficient new separation and purification technologies are widely used.

3. Separation and Purification Technology and Its Application

3.1. Ultrafiltration

Ultrafiltration technology is a new separation technology that emerged in the 1970s. It has low cost and energy consumption, mild operating conditions, and is especially suitable for extraction of active molecules. After the drugs pass through the corresponding extraction steps, effective separation steps are required, and the corresponding separation and purification technologies will be

applied here. Compared with shallow filtration, deep filtration has many tortuous and slender channels. In addition to relying on the surface of filter material to intercept the particles in fermentation broth, it can also intercept finer particles through adsorption. It has the advantages of no relevant transfer, no need to add any chemicals, and can be operated at low temperature. When a substance reaches a certain concentration, the system is divided into two phases. Solutes are separated by different partition coefficients in the two phases. The advantage is that both phases are water and there is no problem of organic solvent residue. In the ultrafiltration mode, ultrafiltration membranes with different pore sizes correspond to the separation operations of substances with different sizes, and mainly separate pharmaceutical components such as enzymes, polysaccharides, antibiotics, proteins and the like. Ultrafiltration membrane technology can effectively purify macromolecular substances in the mixed liquid medicine, so it has wide applicability.

3.2. High performance liquid chromatography

The first step of antibody drug purification is to separate the supernatant from cells, cell fragments and other colloidal substances. And the extracted substance cannot reach a completely pure state. This will inevitably lead to a state of drug efficacy is not prominent, which has a huge loss for enterprises. High performance liquid chromatography (HPLC) has the advantages of high efficiency, high speed and high detection flexibility, and has been widely used in biochemistry, food hygiene detection and other fields. The biggest characteristic is that it can be used to obtain the required active substances after simple treatment in crude extract. Antibody group drugs are antibody drugs screened and developed by antibody group related technologies [6]. Compared with traditional antibody drugs, antibody drugs are characterized by high throughput, integration, informatization and systematization. Humanized monoclonal antibodies used for immune regulation have higher success rate of approval than other anti-tumor monoclonal antibodies or the whole humanized monoclonal antibody population. In combination with a medium with opposite charges, antibody molecules bound to the medium can be eluted by changing the pH or salt concentration of the elution buffer. Dense layer mainly affects the separation efficiency of reverse osmosis membrane. As for porous layer, it mainly plays a supporting role and the separation effect is general.

3.3. Affinity chromatography

When the expression of recombinant protein is in the form of inclusion bodies, high expression is a hot spot in recent scientific research. However, this involves a more complicated process of steps. Only after the above steps can the follow-up work be carried out more effectively. It can be seen that the requirements for gradual and orderly progress are stricter. The range of dye ligands is relatively large, and their binding capacity is also large. Among them, the stability and versatility are very high, and they can be regenerated and are very cheap. Agarose with different cross-linking degrees is used as filler framework, which has good compatibility with antibody protein and low non-specific adsorption. However, due to semi-soft gum, the back pressure is relatively large, which limits the high flow rate. Small molecules enter the pore canal while large molecules flow out with the fluid outside the gel particles. The molecules with large relative molecular mass flow out first and the molecules with small relative molecular mass flow out finally, thus achieving the purpose of separation and purification. Affinity chromatography can directly extract high-purity active substances from crude extract and can be completed only once, which is an operation that cannot be achieved by other separation and purification methods. The purified chimeric antibody was identified by cytology, biochemistry, molecular biology, immunology and other means, and proved to be an engineered antibody with the same specific binding to HER2/p185 highly expressed tumor cells as the original murine monoclonal antibody [7]. Due to its high specificity and concentration effect, it has been widely used to separate a small amount of specific proteins, such as antibodies, from a large number of complex solutions.

4. Biopharmaceutical Equipment

The biopharmaceutical equipment is a very critical existence in the biopharmaceutical industry.

To carry out biopharmaceutical, relevant equipment must be used for operation. Advanced production line equipment is an important symbol of an enterprise's strength and a basic condition for pharmaceutical enterprises to pass GMP certification. The application of wireless temperature inflammation system in biopharmaceutical equipment has the characteristics of high measurement accuracy, good stability, reliable performance, simple operation, etc. Large equipment manufacturers are required to carry out installation, commissioning and confirmation, and formally hand over the equipment operation instructions, drawings, certificates and other documents to the equipment management department for management after passing the verification. Therefore, strengthening the maintenance and management of modern biopharmaceutical equipment can effectively promote the production benefits of modern biopharmaceutical enterprises and ensure the maximization of their economic benefits. For example, the advanced pharmaceutical production technology and the complicated structure of pharmaceutical production equipment need to ensure the good operation of the equipment to make the pharmaceutical process accurate and efficient. The research goal of antibody drugs is to carry out in the direction of all human beings, and to seek more new molecular targets, and to develop highly efficient, non-toxic and side-effect monoclonal antibodies and new dosage forms [8]. Strengthening the monitoring of the production line process is to further improve the product quality and achieve the goals of reducing pollutant emissions, saving energy and increasing efficiency.

Temperature verification in biopharmaceutical equipment is a long-term work, and it is also the basic requirement of GMP regulations for biopharmaceutical equipment. Therefore, it is necessary to continuously supplement and improve the GMP document content and standardized operation procedures for equipment maintenance management, give full play to the working efficiency of production equipment, improve productivity, and meet the needs of vaccine production and scientific research [9]. Figure 1 below shows the wireless temperature verification system equipment. When correcting it, first connect the reader, computer and temperature standard source, place the wireless probe in the reader, and the root system software prompts for operation.

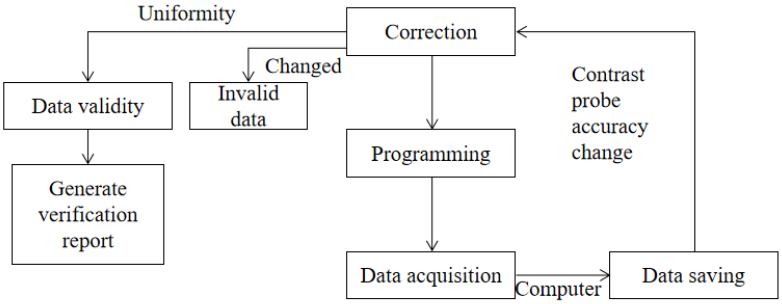

Figure 1 Wireless temperature verification system

In the vaccine production process, automatic production lines are often forced to stop production due to sudden failures, which disrupts the overall production plan of enterprises and delays the delivery time. In the actual working process, the system carries out two-way communication with the computer through special data. Under the operation of the system software, the system can operate on the verification requirements of different users. Equipment purchasing dept. shall purchase equipment according to "equipment demand requisition form", and the bidding office of the enterprise shall submit a bidding application, and the bidding office shall invite bids. Prevent production line equipment from malfunctioning during operation, so as to greatly improve the quality of biological products. Enterprise equipment maintenance management personnel need to formulate a scientific, strict and graded maintenance system for equipment. Compared with the development level of small molecule drugs, our country is very weak in original innovation and has few varieties developed independently. Equipment management and maintenance personnel are required to inspect the operation of equipment in the responsible area every week and record the corresponding inspection results. If any abnormal situation is found, a written explanation shall be made in time. Therefore, to do a good job in equipment maintenance, we must have a high-level

maintenance team to meet the needs of large-scale modern production and ensure the normal operation of the equipment.

The verification report is mainly the most direct manifestation of the running status of biopharmaceutical equipment. The national GMP clearly stipulates that a detailed data report is required for the detection of biopharmaceutical equipment. According to the technical requirements of the equipment for housing, foundation, water, electricity and gas, on-site preparation shall be carried out, and technical personnel from the equipment manufacturer shall be assisted to participate in the installation, commissioning and acceptance of the equipment. In order to improve each employee's management awareness and sense of responsibility in equipment usage, the index management of all employees, all objectives and all processes shall be implemented in equipment management. After completing the maintenance and repair work of the equipment, detailed records shall be made of the equipment maintenance, repair and maintenance contents, faults, troubleshooting and repair results. In order to improve the competitive advantage of enterprises and expand the sales market of enterprises, the stable operation of biopharmaceutical equipment must be guaranteed in the production process of enterprises.

5. Conclusion

To sum up, in biopharmaceuticals, separation and purification technology is a key and basic technology. In general, extraction, separation and purification technology plays an important role in the whole pharmaceutical process. Only through continuous exploration and optimization can the pharmaceutical industry make a breakthrough. After more than 20 years of development, the large-scale antibody drug production and purification process is maturing day by day, and the process development and amplification have transitioned to the mode of combining theoretical guidance with experience. In addition, pharmaceutical equipment is very important for the normal production activities of biopharmaceutical enterprises. In the process of production, enterprises should continuously strengthen the professional operation ability of operators. With the development of computer-aided technology and high-throughput screening technology and the popularization of the technology, the application of separation and purification technology will be more and more extensive, and the low-cost and high-efficiency production of antibodies is just around the corner.

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