THE USE OF MODERN QbD APPROACHES IN THE PHARMACEUTICAL DEVELOPMENT OF TECHNOLOGY OF LIPOSOMAL EYE DROPS

Kruglov Eu. M.[1], Borshchevsky G. I.[2]

[1]National University of Pharmacy of the Ministry of Health of Ukraine, Kharkiv
[2]JSC "Farmak", Kyiv

Introduction

Eye diseases have a significant impact on vision and quality of life. Delivery of drugs to ocular tissues is of great interest, aimed at nanobiotechnological products, in particular liposomal drugs [1, 2].

These drugs, which have a broad spectrum of action, are intensively used for the diagnosis of diseases of various etiologies, prevention and treatment [3, 4, 5]. This widespread usage is due to certain physicochemical properties of liposomes, namely:

- they are fully biodegradable and biocompatible in humans and animals, as derived from natural phospholipids;
- capable of including biologically active substances, including enzymes, hormones, vitamins, antibiotics, immunomodulators, cytostatics, pharmacological drugs and others;
- provide targeted transportation and prolonged release of the included substance;
- included in liposomes substances are more stable, as isolated by the lipid membrane from the destructive effects of environmental factors.

The development of drug technology that ensures reliable and efficient production is one of the essential factors of product quality that ensures success in the pharmaceutical market. The development of technology for the production of nanostructured drugs, which include liposomes, requires a large amount of experimental work focused on research and optimization of technological conditions, validation of processes, selection of auxiliary components [6]. For pharmaceuticals, quality is considered and it must be ensured in full [7, 8]. Scientific approaches such as Quality by Design (QbD) can be used to ensure product quality.

The concept of QbD approach was first proposed several decades ago by Dr. Joseph M. Juran and defines product development and production process to achieve a certain predetermined quality. In his opinion, quality should be built into the product and the way it is produced. Dr. Juran believed that the quality of the product could be planned, and the biggest disadvantages stem from how the quality of the product was planned [9, 10].

In 2002, the FDA announced a new initiative called "Current Good Manufacturing Practices for the 21st Century", which motivates the pharmaceutical industry to use modern QbD-based quality management techniques. Thus, this model begins at the stage of the concept of drug development and is used throughout the development procedure [11, 12].

It should be recognized that the best way to achieve high quality is to embed the quality of the drug at every stage of development, from ingredient selection, screening and justification of the formula, to scaling and adjustment of production processes, including technological optimization.

The incorporation of drug quality begins with the definition of a list of quality requirements, called the quality target product profile (QTPP). ICH Q8 defines QTPP as a "promising summary of the quality characteristics of a medicinal product that will ideally be achieved to ensure the desired quality taking into account the safety and efficacy of the medicinal product". These quality requirements are called quality attributes, and to accurately characterize the various components of QTPP, ie physicochemical properties, it is necessary to understand which of them can potentially be critical quality indicators (CQA) of the formulation. The definition of IQ Q8 for CQA is a "physical, chemical, biological or microbiological property or characteristic that must be within the appropriate limits, range or distribution to ensure the desired product quality". To develop a final product with the desired CQA, quality must be considered in the product based on an understanding of critical material attributes (CMA) and critical process parameters (CPP), concepts developed by the QbD approach [7].

QTPP for drugs in the form of eye drops is a potential list of desirable quality indicators that should be present in the final product [7, 12]. This may include elements such as dosage form, way of administration, particle size or globules, rheological behavior, drug concentration, homogeneity, pH, release and penetration of the drug *in vitro*, microbial limits etc. They can be controlled and optimized in the QbD process to obtain the desired finished product [1, 13, 14].

All factors, including equipment, technology transfer, production variables and QTPP, should be considered to develop the optimal production process. Variations of the proposed production processes are considered risk factors that may affect the critical quality of the drug during storage [15, 16]. The likelihood and potential difficulty of these risk factors and the consequences of failed regimens should be determined to develop CMA and CPP action plans that will help avoid risk factors [17].

The aim of the work was to propose the technology of obtaining liposomal eye drops on the basis of experiment according to the QbD approach and to evaluate the influence of critical process parameters (CPP) on critical quality indicators (CQA) of the obtained intermediates.

Materials and Methods

Lipids ("Lipoid", Germany) were used to make liposomes. Crystalline glycine, edetate disodium, sodium chloride, sodium hydroxide, hydrochloric acid were used (Sigma-Aldrich, USA). Value pH was monitored on a pH-meter Seven Compact (Mettler Toledo, USA). Method of high pressure extrusion, which was performed on the Microfluidiser M-110P (Microfluidics, USA) used for homogenization. The size of the liposomes was determined at 20 °C on a Zetasizer Nano ZS (Malvern Instruments, UK). The level of

DOI: 10.5281/zenodo.6350205

encapsulation of the peptide complex in liposomes, the concentration of the peptide complex, the content of impurities were monitored by high performance liquid chromatography (HPLC) using Agilent 1200 chromatographs (USA).

Results and discussion

The development of the technological process of drug preparation was based on previous experience in the manufacture of drugs in the form of aqueous solutions (eye drops, nasal solutions, solutions for injection, syrups, spray). Data on the physicochemical characteristics of APIs and excipients included in the composition of drug were used. The following was taken into account during the development of the technological process:

1. The components of the drug (except phosphatidylcholine) are easily soluble in water;

2. According to API data, the concentrate of the deproteinized dermal layer of pig skin has good stability in the pH range from 4.0 to 6.0;

3. All components of the drug are compatible and do not cause degradation of each other.

Given the fact that eye drops are an aqueous solution of API and excipients for the study of the production process, the following stages were provided:

1. Preparation of liposome suspension.
2. Obtaining the drug.
3. Filtration.
4. Filling of bottles (packing).

Stage "Preparation of liposome suspension" consists of the following technological operations: dissolution of phosphatidylcholine, obtaining a suspension of liposomes with a size of 120-140 nm.

Stage "Obtaining the drug" consists of the following technological operations: the inclusion of protein concentrate in liposomes, dissolution of excipients, pH adjustment. The production process is developed based on the capabilities of technological equipment of JSC "Farmak" (Kyiv).

Scheme of the technological process of obtaining liposomal eye drops is represented in fig. The development of the technology took place at the laboratory level with the subsequent reproduction (scaling) of the optimal technology in the shop conditions.

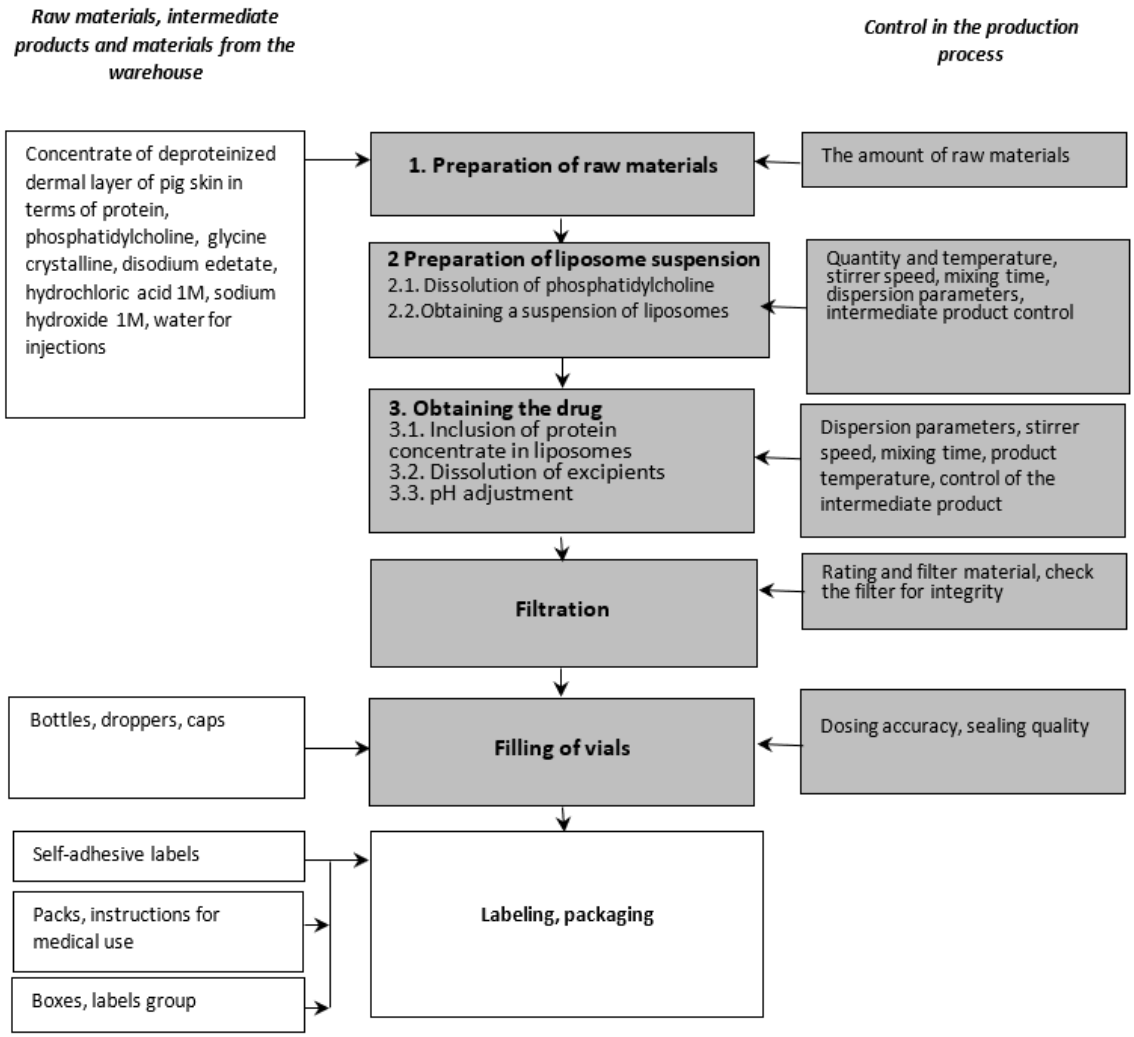

Fig. Scheme of the technological process of obtaining liposomal eye drops

DOI: 10.5281/zenodo.6350205

Risk analysis according to ICH Q9 was used to determine which variables and single operations are likely to affect the quality of intermediates and the drug. The initial risk assessment of the production process of liposomal eye drops presents in table.

Table. Initial risk assessment of the production process of liposomal eye drops

Changeable and individual operations	Critical quality indicators of the finished medicinal product		
	Quantitative content of API	Impurities	MBP
Preparation of liposome suspension:			
Dissolution of phosphatidylcholine	high	low	low
Obtaining a suspension of liposomes with a size of 120-140 nm	high	low	low
Inclusion of protein concentrate in liposomes	high	low	low
Dissolution of excipients	low	low	low
pH adjustment	low	low	low
Filtration	high	high	high
Filling of vials	high	high	low

On the basis of risk analysis at all stages of the technological process, potential risks for the critical quality indicators of the finished medicinal product (FMP) are identified. However, by controlling parameters and technological process, risks can be reduced to an acceptable level.

All stages of the process were studied empirically and the interactions between the variable parameters of the technology were analyzed. Key factors involved in manufacturing operations were examined for risks assessment. All possible factors were identified, analyzed and evaluated. Most of the factors were either non-critical or controlled in the process.

Based on statistical modeling, a flexible and reliable technology of liposomal eye drop has been developed. The optimal parameters of the technological stages of the production process, which are given below, have been established.

During the development of the technological operation “Preparation of liposome suspension” based on the risk analysis of this process, the potential risk for the indicator was quantified.

At this stage, the primary dispersion of soybean phosphatidylcholine obtained in the previous stage takes place before the inclusion of the protein of the deproteinized dermal layer of pig skin.

The particle size of phosphatidylcholine sufficient for the transition to the next stage was determined empirically: in 1 L of phosphatidylcholine solution of soybeans of different sizes was included protein deproteinized dermal layer of pig skin at a working pressure of 20000 PSI for 20 min controlled percentage of protein inclusion.

Complete inclusion of protein deproteinized dermal layer of pig skin in 1 L of liposome solution at a working pressure of the dispersant 20000 PSI for 20 minutes occurs in solutions of liposomes with a size of 120-140 nm and less. Since to obtain smaller liposomes it is necessary to disperse for a longer time, the most optimal from a technological point of view is a solution with a liposome size of 120-140 nm for the transition to the next stage.

During the development of the technological operation “Inclusion of protein concentrate in liposomes” based on risk analysis, the potential risk for the indicator was quantified.

During pharmaceutical development, criteria for the particle size of the developed drug were established: the first peak is not more than 100 nm, the second peak is not more than 400 nm, if the particle size does not meet these criteria, and then the solution clogs the filter pores with 0.22 μm and cannot be filtered at the stage filtration.

During the development of the technological operation “Dissolution of excipients” on the basis of a preliminary risk analysis, it was found that at this stage the risks for the finished drug are low. The only parameters of the technological process at this stage, which must be selected for the complete dissolution of auxiliary substances, were the speed and time of mixing. A flexible and reliable production operation was developed based on statistical modeling.

While development of the technological operation “pH adjustment” taking into account the effect of pH on the concentrate of deproteinized dermal layer of pig skin, found no risk of exposure in the range of pH from 4.0 to 5.0.

Based on previous experience using sodium hydroxide solution and hydrochloric acid to adjust the pH, 1M sodium hydroxide solution and diluted hydrochloric acid solution were used. Adding of 1M sodium hydroxide solution and diluted hydrochloric acid was performed in workshop conditions.

During the development of the “Filtration” stage, based on the risk analysis at this stage, the potential risk for the quantitative content of API, impurities and MBP was identified. To minimize microbial contamination of the drug during storage, a filter with a pore size of 0.2 μm was selected for filtration.

To select the filter material, Ultipor N66 0.2 μm filters (nylon membrane material) manufactured by Pall, Fluorodyne II 0.2 μm (polyvidone fluoride membrane material) manufactured by Pall, Propor PES 0.2 μm (membrane material polyester sulfone) manufactured by DomnickHunter, Sartopore 2 0.2 μm (membrane material

DOI: 10.5281/zenodo.6350205

polyester sulfone) manufactured by Sartirius were investigated.

The prepared solution of the drug was passed through the studied filters, the sorption of API on the membrane and the throughput were monitored. All tested filters do not show sorption properties, but the Sartopore 2 0.2 μm filter (membrane material polyester sulfone) manufactured by Sartorius has better throughput, so this type of filter was used for sterilizing filtration.

During the development of the stage "Filling vials (packaging)" based on the risk analysis at this stage, potential risks were identified. According to the results of three validation series, it was proved that the use of standard process parameters in the workshop conditions meets the established requirements. Production sites of JSC "Farmak" are GMP certified. The process of filling vials took place automatically with minimal operator intervention. All stages of filling vials are validated and meet GMP requirements.

Conclusions

1. An original experimental industrial technology for producing liposomal form of eye drops has been proposed.
2. While developing the technological process, risks of each unit of operation were considered. Experimental studies have been identified and performed with the aim of establishing additional scientific knowledge and understanding to reduce risks to an acceptable level. Following the experiments, the initial risk assessment of the technological process has been updated.
3. The developed technology was applied to obtain liposomal eye drops for clinical trials.
4. The proposed technology makes it possible to obtain a stable drug with a predetermined quality according to modern quality management methods based on QbD approach, which ensures the therapeutic effect of the drug for further clinical trials and gives it advantages in the pharmaceutical market among analog drugs.

The use of modern QbD approaches in the pharmaceutical development of technology of liposomal eye drops

Kruglov Eu. M., Borshchevsky G. I.

Introduction. Delivery of drugs to ocular tissues is of great interest, aimed at nanobiotechnological products, in particular liposomal drugs. The development of technology for the production of nanostructured drugs, which include liposomes, requires a large amount of experimental work focused on research and optimization of technological conditions, validation of processes, selection of auxiliary components. The **aim of the work** was to propose the technology of obtaining liposomal eye drops on the basis of experiment according to the QbD approach and to evaluate the influence of critical process parameters (CPP) on critical quality indicators (CQA) of the obtained intermediates. **Materials and methods.** Lipids were used to make liposomes. Value pH was monitored on a pH-meter Seven Compact (Mettler Toledo, USA). Method of high pressure extrusion, which was performed on the Microfluidiser M-110P (Microfluidics, USA) used for homogenization. The size of the liposomes was determined at 20 °C on a Zetasizer Nano ZS (Malvern Instruments, UK). The level of encapsulation of the peptide complex in liposomes, the concentration of the peptide complex, the content of impurities were monitored by high performance liquid chromatography (HPLC) using Agilent 1200 chromatographs (USA). **Results and discussion.** Based on statistical modeling, a flexible and reliable technology of liposomal eye drop has been developed. The optimal parameters of the technological stages of the production process have been established. All stages of the process were studied empirically and the interactions between the variable parameters of the technology were analyzed. Key factors involved in manufacturing operations were examined for risks assessment. All possible factors were identified, analyzed and evaluated. Most of the factors were either non-critical or controlled in the process. **Conclusions.** While developing the technological process, risks of each unit of operation were considered. Experimental studies have been identified and performed with the aim of establishing additional scientific knowledge and understanding to reduce risks to an acceptable level. Following the experiments, the initial risk assessment of the technological process has been updated.

Keywords: eye drops, technology, liposome, quality.

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DOI: 10.5281/zenodo.6350205

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DOI: 10.5281/zenodo.6350205