Justification for the Choice of Excipients of Injectable Solution Based on Essential Phospholipids

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Abstract. The article addresses the justification for the choice of excipients of injectable solution based on essential phospholipids. The main physicochemical and microbiological quality attributes of the liposomal product Lesfal are presented. It was demonstrated that they comply with the requirements of the State Pharmacopoeia of Ukraine, European Pharmacopeia and the reference product

Keywords excipients, injectable solution, essential phospholipids, quality attributes, liposomes.

Introduction. The progress in synthesis and improvement of methods of extraction of biologically active compounds from animal and herbal substances that result from the science advancement rate offers multiple possibilities for improvement of public drug supply. Using the advances in biotechnology and nanotechnology, the contemporary pharmaceutical science can produce medicinal products delivering the active substances to the affected area of a macroorganism in precisely adjusted quantities [8].

The paramount task during nanobiotechnology based manufacture of medicinal products is to produce dosage forms, which would both preserve the substance potency and its active effect on the entire human body. The solution of this task today preconditions the choice of an optimum dosage form for a specific active substance and the process for its production [1, 7].

Liposomes keep their encapsulated active substances intact preventing them from plasma protein binding, destruction by ferments, as well as reducing a possibility of immune and other systemic reactions of the body to the substances, which are administered with liposomes, as they do not penetrate through the external liposome lipid layer into the blood. At that, the effect of the active substances contained in the liposomes is considerably prolonged due to their slow release [5, 6].

As a rule, the liposomes administered intravenously bind with the organs of the reticuloendothelial system, mainly with the liver and spleen [9].

This paper presents the studies on justification of formulation of an injectable liposomal medicinal product based on essential phospholipids for treatment of liver diseases [2].

The aim of the work – justification for the choice of excipients of injectable solution based on essential phospholipids.

Materials and methods. The medicinal product Essential Phospholipids (Lesfal), solution for injection 5.0 mL in ampoules No. 5 was developed as a generic drug of the original product Essentiale N, solution for intravenous injections 5.0 mL in ampoules No. 5, manufactured by A. Nattermann & CiE GmbH for Aventis Pharma Deutschland GmbH, Germany (4).

The following excipients were used in the activities: benzyl alcohol, deoxycholic acid, sodium chloride, sodium hydroxide, riboflavin, water for injections.

The physicochemical studies (description, identification, clarity, colour, relative density, particulate matter, extractable volume, assay, sterility) of the drug samples were performed in accordance with the methods of the State Pharmacopoeia of Ukraine [10], the test for bacterial endotoxins was performed per European Pharmacopoeia [11].

pH was determined using the Mettler Toledo SevenCompact pH meter. Chromatographic studies were performed using Agilent 1100 LCMSD SL serial chromatograph (diode array detection) equipped with analytical chromatographic column Eclipse XDB C18 (4.6 mm * 150 μm), eluent: gradient acetonitrile - 1% trifluoroacetic acid aqueous solution.

Results and discussions. During the formulation development of Essential Phospholipids, solution for injection 5.0 mL in ampoules #5, the impact of various factors on the product formulation stability was studied as well as its compliance with the specified quality attributes of the reference product (physicochemical properties of the active substance and excipients, process parameters of the product manufacturing process (time, temperature, pH, filtration conditions, etc.)).

The physicochemical properties of Essential Phospholipids, solution for injection 5.0 mL in ampoules No. 5, were studied in comparison with Essentiale N, solution for intravenous injections 5.0 mL in ampoules No. 5 manufactured by A.Nattermann & CiE GmbH for Aventis Pharma Deutschland GmbH.

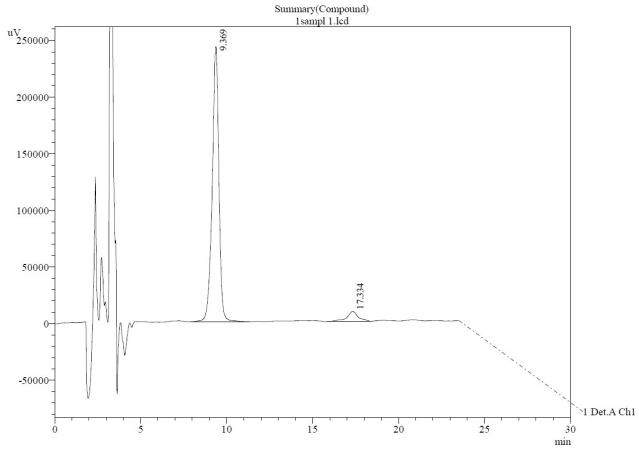
Essential Phospholipids® = EPL® - natural choline phosphoric acid diglyceride esters, soybean extract with high content of unsaturated fatty acids, mainly of linoleic (about 70 %), linolenic and oleic acids, are used as the main active substance in the formulation of the reference product Essentiale N, solution for intravenous injections 5.0 mL in ampoules No. 5. The description of the formulation of Essential Phospholipids® = EPL® from soybeans is reported in the

literature (Lipostabil booklet by Rhone - Poulenc Rorer, Nattermann, International GMBH. Germany) D. LeKim и H. Betzing (1974).

During the development of the formulation of Essential Phospholipids (Lesfal), solution for injection 5.0 mL in ampoules No. 5, the substance LIPOID S100 – high purity soybean lecithin with phosphatidylcholine content of not less than 94.0 % was chosen. The fatty acid content of LIPOID S100 is given below (% of the total quantity of fatty acids):

- Palmitic acid 12 - 17 %
- Stearic acid 2 - 5 %
- Oleic acid 7-12 %
- Linolenic acid 59 - 70 %
- Linolenic 5 - 8 %

During the comparative study of physicochemical properties of the developed product Lesfal, solution for injection 5.0 mL in ampoules #5, and the reference product Essentiale N, solution for intravenous injections 5.0 mL in ampoules No. 5, the identity of their active substances was demonstrated. Thus, during the identification and assay of 3-sn-Phosphatidylcholine by HPLC, the retention time of the major peak in the chromatogram obtained with the test solutions was similar to the retention time of the phosphatidylcholine peak in the chromatogram obtained with the reference solution with an accuracy of ± 2 % (Figures 1, 2).



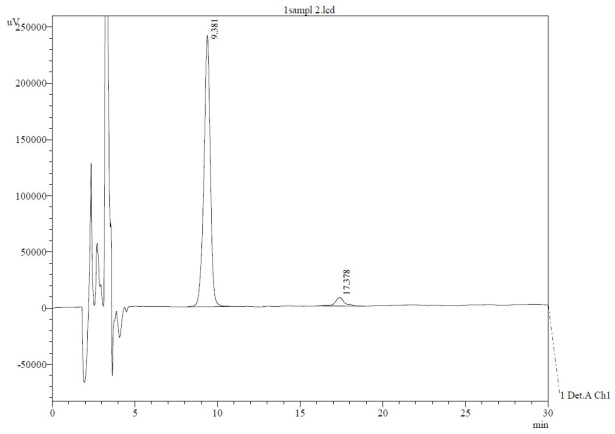
<< Detector A >>
ID#1 Compound Name: RT9.369

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
1sampl 1.lcd	1sampl		9.369	6562970	240041	0.000
1sampl 2.lcd	1sampl		9.381	6467104	238402	0.000
Average			9.375	6515037	239222	0.000
%RSD			0.087	1.040	0.485	0.000
Maximum			9.381	6562970	240041	0.000
Minimum			9.369	6467104	238402	0.000
Standard Deviation			0.008	67787	1159	0.000

Fig. 1. Chromatogram of Essentiale

The comparative study of the degree of fatty acid unsaturation using the iodine test method demonstrated close results in the reference product and the developed product Lesfal based on the LIPOID S100 substance (iodine value – 61.45).

During the development of the formulation of Lesfal, solution for injection 5.0 mL in ampoules No. 5, the following excipients were selected: benzyl alcohol, sodium chloride, sodium hydroxide, deoxycholic acid, riboflavin, water for injections. Their functions are given in Table 1. These substances are used in the reference product Essentiale N, solution for intravenous injections 5.0 mL in ampoules No. 5.



ID#2 Compound Name: RT17.334

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
1sampl 1.lcd	1sampl		17.334	404812	8591	0.000
1sampl 2.lcd	1sampl		17.378	326800	7723	0.000
Average			17.356	365806	8157	0.000
%RSD			0.177	15.080	7.522	0.000
Maximum			17.378	404812	8591	0.000
Minimum			17.334	326800	7723	0.000
Standard Deviation			0.031	55163	614	0.000

Fig. 2. Chromatogram of Lesfal

Physicochemical characteristics of the developed product Lesfal were the criterion of evaluation of the quantitative selection of excipients (Tables 2, 3, 4).

Benzyl alcohol in the reference product is used as an antimicrobial preservative in the amount of 9 mg/mL (42.8 to 47.3 mg per 5 mL). The selected amount of benzyl alcohol maintains the stability of the dosage form throughout its shelf life [4].

Table 1: Functions of the Excipients Formulated into Lesfal

Name of Component	Functional Purpose
Benzyl alcohol (United States Pharmacopoeia*)	Antimicrobial preservative
Deoxycholic acid (Analytical Normative Documents of Farmak JSC)	Emulsifier
Sodium chloride (European Pharmacopoeia*)	Osmotic component
Sodium hydroxide (State Pharmacopoeia of Ukraine*)	Salt-forming agent
Riboflavin (European Pharmacopoeia*)	Colouring agent
Water for injections (State Pharmacopoeia of Ukraine*)	Solvent

Sodium chloride has been formulated into Lesfal as an osmotic component. The selected quantity of sodium chloride ensures the required osmolarity values and chloride content corresponding to the reference product.

Sodium hydroxide performs the function of the deoxycholic acid salt formation. With the specified sodium hydroxide value the qualitative composition and physicochemical properties (pH, osmolarity, content of sodium and chlorides) correspond to the reference product and the specified requirements to Lesfal.

Deoxycholic acid performs the function of an emulsifier of essential phospholipids. Deoxycholic acid is not soluble in water, however, soluble in ethyl alcohol and chloroform. In the course of development it was revealed that deoxycholic acid precipitates with pH less than 5. During the development of the qualitative and quantitative composition

of Lesfal the quantity of deoxycholic acid, which creates the required physicochemical attributes (pH, osmolarity, content of sodium and chlorides, particle size) was selected.

Riboflavin performs the function of a colouring agent. During the development of the qualitative and quantitative composition of Lesfal, solution for injection 5.0 mL in ampoules No. 5, the quantity of riboflavin was selected, which preconditions standard physicochemical properties (appearance, colour) (3).

Water for injections is used during the dissolution and dispersion of the substances formulated into the product. During the development of the qualitative and quantitative composition of Essential Phospholipids, solution for injections 5.0 mL in ampoules No. 5, the quantity of water for injections was selected according to the developed composition of the active substance and excipients (2).

The physicochemical properties are given in Tables 2, 3, 4.

Table 2: Study of Physicochemical Properties of Lesfal Essential Phospholipids, Solution for Injections 5.0 mL in Ampoules No. 5

Quality Attributes	Specifications of Normative Documents	Study Results
Appearance	A clear, yellow-green solution	Complies
Colour	-	Yellow
Clarity	The product must pass the tests in comparison with reference suspension I	Complies
Colour	Greenish-yellow colour. The product should be not more intensively coloured than the reference solution GY.	Complies
Particulate matter Visible particles Subvisible particles	The product should comply with the tests Particles ≥10 μm – not more than 6,000/ampoule Particles ≥25 μm – not more than 600/ampoule	Complies
pH	7.5 to 9.5	8.76
Relative density	1.007 to 1.012	1.008
Extractable volume	Not less than 5.0 mL	Complies

Table 3: Identification of Active Substances of Lesfal

Identification	Specifications of Normative and Technical Documents	Study Results
3-sn-Phosphatidylcholine, HPLC	The retention time of the major peak in the chromatogram obtained with the test solution for the Assay of Phosphatidylcholine should correspond to the retention time of the peak of phosphatidylcholine in the chromatogram obtained with the reference solution with an accuracy of ± 2%.	Complies
3-sn-Lysophosphatidylcholine, HPLC	- The retention time of the major peak in the chromatogram obtained with the test solution for the Assay of Ethanol should correspond to the retention time of the peak of ethanol in the chromatogram obtained with the reference solution with an accuracy of ± 2%.	Complies
Ethanol, gas chromatography		Complies
Benzyl alcohol, HPLC	The retention time of the major peak in the chromatogram obtained with the test solution for the Assay of Benzyl Alcohol should correspond to the retention time of the peak of benzyl alcohol in the chromatogram obtained with the reference solution with an accuracy of ± 2%.	
Riboflavin	Bright yellow fluorescence	Complies

Table 4: Assay of Active Substances of Lesfal

Substance	Specifications of Normative and Technical Documents	Study Results
3-sn-Phosphatidylcholine, HPLC		
At release	52.26 to 57.76 mg/mL	54.96 mg
During shelf-life	45.0 to 57.76 mg/mL	54.95
3-sn-Lysophosphatidylcholine, HPLC mg per 5 mL		
At release	Not more than 4 mg/mL	2.09
During shelf-life	Not more than 6.62 mg/mL	2.11
Benzyl alcohol, HPLC		
At release	8.55 to 9.45 mg/mL	9.03
During shelf-life	8.10 to 9.45 mg/mL	9.03
Ethanol, gas chromatography	Not more than 5.02 mg/mL	4.50
Bacterial endotoxins (European Pharmacopoeia)	Not more than 31 EU/mL	≤5 EU/mL
Content of chlorides	-	2.3 mg/mL
Content of sodium	-	2.995 g/L

As shown by the presented data the main physicochemical and microbiological quality attributes of the medicinal product comply with the requirements of the State Pharmacopoeia of Ukraine.

Conclusions.

1. The studies were performed to justify the choice of excipients of the liposomal injectable solution based on essential phospholipids.
2. Based on the physicochemical and microbiological experimental data, the composition of the medicinal product Essential Phospholipids (Lesfal), solution for injection 5.0 mL in ampoules #5, was justified; the product complies with the specified physicochemical and microbiological attributes on a batch-to-batch basis.

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