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Y. V. Bovsunovska[1,2], V. V. Rudiuk[2], N. V. Harna[1], O. S. Holovchenko[1], V. A. Georgiyants[1]

[1] National University of Pharmacy of the Ministry of Health of Ukraine,
53 Pushkinska str., 61002 Kharkiv, Ukraine
[2] JSC Farmak, 63 Kyrylivska str., 04080 Kyiv, Ukraine

Obtaining the Enoxaparin Sodium Substance Equivalent to the Original Clexane® and Lovenox®. The Selection of Technological Parameters and Optimization of the "Greenness" of the Purification Stage

Abstract

The aim of the study was to adjust and optimize the purification stage of crude enoxaparin sodium to obtain a substance equivalent to the original drugs Clexane® and Lovenox® according to the criteria specified by the FDA. The purification stage involves the reprecipitation of crude enoxaparin in methanol. Determining the ratio of solvents required for the reprecipitation is important for studying the correlation between the experimental conditions of the technological process and the structural characteristics of enoxaparin samples. In the study, the method of purification of enoxaparin sodium described in the patent was assessed, and the following variations of the MeOH:H_2O solvent ratio were selected – 4:1; 2:1; 1:1. The obtained samples of enoxaparin sodium were analyzed according to the in-house specification developed on the basis of the pharmacopoeial monograph, as well as by non-pharmacopoeial methods, such as two-dimensional NMR spectroscopy (HSQC) and size exclusion chromatography (SEC) for detailed characterization of the molecule. Strategies of greening of the enoxaparin sodium purification stage by reducing the E-factor were also considered in the study. Considering the principles of "green" chemistry, the method of purification of crude enoxaparin sodium was optimized by the solvent regeneration. It was experimentally possible to demonstrate the effect of the solvent ratio at the stage of purification of crude enoxaparin on the composition, as well as on the number and distribution of oligosaccharide fractions in the molecule. Based on the results of the study, it can be concluded that the ratio of MeOH:H_2O=1:1 allows obtaining samples that are closest to Clexane® and Lovenox® in terms of the molecular weight distribution profile and the composition profile. The E-factor was also reduced from 14 to 5.25 by solvent regeneration.

Keywords: enoxaparin sodium; low-molecular-weight heparin; technological parameters; compositional analysis; HSQC; size-exclusion chromatography; green chemistry; E-factor; solvent regeneration

Ю. В. Бовсуновська[1,2], В. В. Рудюк[2], Н. В. Гарна[1], О. С. Головченко[1], В. А. Георгіянц[1]

*[1] Національний фармацевтичний університет Міністерства охорони здоров'я України,
вул. Пушкінська, 53, м. Харків, 61002, Україна*
[2] АТ «Фармак», вул. Кирилівська, 63, м. Київ, 04080, Україна

Одержання субстанції еноксапарину натрію, еквівалентної оригінальним Clexane® та Lovenox®. Підбір технологічних параметрів та оптимізація «зеленості» стадії очищення

Анотація

Метою роботи було налаштувати та оптимізувати стадію очищення технічного еноксапарину натрію для отримання субстанції, еквівалентної оригінальним препаратам Clexane® та Lovenox® за критеріями, окресленими FDA. Стадія очищення передбачає переосадження неочищеного еноксапарину із метанолу. Визначення необхідного співвідношення розчинників для переосадження є важливим для дослідження кореляції між експериментальними умовами технологічного процесу та структурними характеристиками зразків еноксапарину. У дослідженні було оцінено спосіб очищення еноксапарину натрію, описаний у патенті, і обрано такі варіанти співвідношення розчинників MeOH:H_2O – 4:1; 2:1; 1:1. Отримані зразки еноксапарину натрію аналізували відповідно до внутрішньої специфікації, розробленої на основі фармакопейної монографії, а також за допомогою нефармакопейних методів, таких, як двовимірна ЯМР-спектроскопія (HSQC) та ексклюзійна хроматографія (SEC) для детальної характеристики. молекули. У дослідженні також розглядали стратегії екологізації етапу очищення еноксапарину натрію шляхом зниження Е-фактора. З огляду

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на принципи «зеленої» хімії метод очищення неочищеного еноксапарину натрію було оптимізовано шляхом регенерації розчинника. Експериментально вдалося продемонструвати вплив співвідношення розчинників на стадії очищення неочищеного еноксапарину на склад, а також на кількість і розподіл фракцій олігосахаридів у молекулі. За результатами дослідження можна зробити висновок, що співвідношення MeOH:H_2O = 1:1 дозволяє отримати зразки, які за профілем молекулярно-масового розподілу та профілем складу найбільш наближені до Clexane® та Lovenox®. E-коефіцієнт також було знижено з 14 до 5,25 шляхом регенерації розчинника.

Ключові слова: еноксапарин натрію; низькомолекулярний гепарин; технологічні параметри; композиційний аналіз; HSQC; ексклюзійна хроматографія; «зелена» хімія; E-фактор; регенерація розчинника

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■ Introduction

Enoxaparin sodium is a modern low-molecular semi-synthetic anticoagulant, which is a product of the multi-stage conversion of sodium heparin [1]. Enoxaparin sodium has the same properties as its precursor heparin sodium, but due to its improved structure, it does not have the side effects typical of heparin caused by its complex structure and very high molecular weight [2, 3].

Enoxaparin sodium is a heterogeneous mixture of oligosaccharides with a complex structure consisting of repeating units of disaccharide building blocks with one glucuronic acid (GlcA) or iduronic acid (IdoA) residue and one glucosamine (GlcN) residue, which is either *N*-sulfated (GlcNS), or *N*-acetylated (GlcNAc), linked by glycosidic bonds. Enoxaparin sodium is characterized by unique structural elements (fingerprints) that are formed because of modifications during depolymerization, namely, such structures as 4,5-uronates at non-reducing ends and 1,6-anhydro structures at reducing ends [4, 5]. Enoxaparin sodium is a substance of biological origin, i.e., isolated from animal tissues and differs from "normal" substances by its high molecular weight and complex heterogeneous structure, which complicates the development and introduction of similar drugs to the market. Due to the complexity of the structure and the previous experience during the heparin crisis [6], there are serious discussions in the world on the issues of proving the equivalence of generic low-molecular-weight heparins (LMWHs) and establishing permissible fluctuations of the "norm" of biochemical and biological indicators, which potentially affect the safety and effectiveness of the drug. As a result, EMA and FDA have initiated guidelines to confirm the similarity of LMWH [7, 8]. The FDA, for example, introduced a scientific approach to demonstrate the equivalence of generic LMWHs to references, which included compliance not only with biological, but also with chemical characteristics, such as the sequence of disaccharide building blocks, the sequence of oligosaccharide fragments, etc. [9]. Since the aim of our work was the synthesis of the Enoxparin molecule demonstrating the equivalence to the original *Clexane*® and *Lovenox*® (Sanofi-Aventis) according to the specified FDA criteria [10], we conducted a large study to adjust the technological parameters of the process at each stage to obtain a substance as close as possible to the originator [11]. The methods described in the patent [12] were taken as a basis. The analysis of samples for comparison was carried out according to the internal specification developed based on the pharmacopoeial monograph, as well as according to specific methods. Since the structure requires accurate, painstaking analysis of saccharide units and their sequence, additional methods of analysis of similar structures were introduced [13, 14].

One of the steps in the synthesis of enoxaparin is the purification stage, which is a very important in achieving the equivalence with the original *Clexane*® and *Lovenox*® (Sanofi-Aventis). Purification of the substance involves decolorization of enoxaparin sodium, pH correction, elimination of degradation products after depolymerization, and correction of the molecular composition. There are many different methods for the purification of enoxaparin, for example, lyophilization of enoxaparin sodium solution, decolorization of the solution with hydrogen peroxide,

followed by reprecipitation using carbon filters, ion exchange resins, etc. [15]. In the experiment described in this article, the solution reprecipitation was used as a purification method. It is also known that the amount of the solvent for reprecipitation of enoxaparin affects the number and distribution of short and long saccharide chains in the molecule, so we focused on this. Decolorization of the solution is also an important component of obtaining API of proper quality, but it is not a priority of this experiment.

Compliance with the principles of "green" chemistry is no less important in the development of synthetic technologies. "Green" chemistry is a direction in modern chemistry that consists in the improvement of technologies regarding the effective use of the raw material and energy, the avoidance of toxic and poisonous substances, the reduction of waste or the repeated use of chemicals and materials [16]. In this study, the E-factor was chosen as the accent metric for the analysis of "greenness". The E-factor is the ratio of the amount of waste to the amount of a product. All raw materials used, except water, are included in the calculation. The higher the value of the E-factor, the greater the amount of waste [17]. One of the tasks of this work was also to minimize waste at the stage of enoxaparin sodium purification, thereby improving the "greenness" of the synthesis of enoxaparin sodium.

■ The Research Methodology

In the process of planning the experiment for the purification of crude enoxaparin sodium, an analysis of the methods described in the literature was performed.

Thus, the patent [18] describes the following protocol, which it was decided to use as a basis, but with a change in the amount of methanol to observe the effect on the structure of the molecule: "*Suspend crude enoxaparin sodium (5 g) in 50 mL of purified water and dissolve. Add 5 g of sodium chloride and mix. The product is precipitated by adding 150 mL of methanol, filtered, and dried under vacuum at 55°C for 9 hours, yielding 4.39 g of enoxaparin sodium*".

The study included tasks outlined below.

1. To purify the substance according to the parameters selected and to study the effect of the solvent ratio on the product composition in order to obtain a substance equivalent to the original *Clexane*® and *Lovenox*®. The following variants of the ratio of MeOH:H_2O were considered: 4:1; 2:1; 1:1.

1.1. First, it was decided to conduct a test experiment to understand the general trend of the effect of the solvent ratio on the composition and distribution of low- and high-molecular fractions of enoxaparin sodium. Thus, crude enoxaparin sodium synthesized under the so-called "standard conditions" [11] (base/ester ratio – 0.07; the reaction mass temperature – 62°C; the reaction time – 1 hour) was purified in two ways with the following solvent ratios:

- MeOH:H_2O = 4:1 (D475);
- MeOH:H_2O = 1:1 (D478).

1.2. The next step was to analyze the results obtained for more accurate processing of the purification stage, including reprecipitation of samples of crude enoxaparin synthesized according to the optimized parameters of the depolymerization stage [11] (alkali/benzyl ester of the heparin ratio – 0.06; temperature – 57°C, the holding time of the reaction mixture – 1.5 (D492 and D493) and 2 hours (D494 and D495) in the ratio of:

- MeOH:H_2O = 2:1 (D492 and D494);
- MeOH:H_2O = 1:1 (D493 and D495).

2. To optimize the method of synthesis and purification of enoxaparin sodium in view of the principles of "green" chemistry.

The reprecipitation stage is carried out at atmospheric pressure and room temperature, which does not contradict the principles of "green" chemistry. Methanol, which is a poisonous substance, is used as a precipitating agent. However, it is worth noting that according to the in-house guidelines for the selection of solvents of several pharmaceutical companies, methanol belongs to the category "to be confirmed" (**Table 1**) [19].

In addition, the use of methanol is justified by the possibility of obtaining a crystalline precipitate, while the use of solvents with less harmful environmental effects, such as ethanol or isopropanol, provokes the formation of a finely dispersed suspension, which makes it impossible to isolate the precipitate of the substance. One of the most important indicators of "green" chemistry is the E-factor, which is a method of measuring and regulating the amount of waste.

Table 1. Generalized comparison of solvent ratings

Solvent	Astra Zeneka	GCI-PR	GlaxoSmithKlein	Pfizer	Sanofi	Total
MeOH	19	14	14	Preferably	Recommended	To be confirmed

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The E-factor is the actual amount of waste defined as "everything except the desired product" produced per kg of the product, including the loss of solvents and chemicals used in processing [20]. In pharmaceutical production, solvents account for 80–90% of the total mass of non-aqueous material used, most of the waste generated, and 75–80% of the environmental impact of the life cycle, creating the need for solvent regeneration with the subsequent reuse. Therefore, the greening of this stage was carried out due to the regeneration of methanol, which affected the reduction of the amount of waste, and, as a result, a decrease in the E-factor indicator.

3. After analyzing the results of the experimental studies, to make corrections in the method of the enoxaparin sodium synthesis.

■ Results and discussion

As mentioned earlier, the aim of this work was to study the effect of solvents on the composition of the enoxaparin substance. The experiment was conditionally divided into two stages described below.

1. For the study, we chose samples of crude enoxaparin sodium that were processed according to "standard" non-optimized technological parameters using the methodology in the patent [12] and those samples that were processed according to optimized parameters. Crude enoxaparin sodium obtained under the so-called "standard conditions" was purified by reprecipitation of an aqueous solution of enoxaparin in methanol. The ratios of water and methanol for reprecipitation were chosen rather roughly, 4:1 and 1:1, for the initial assessment of the effect of solvents on the distribution of saccharide fractions with different molecular weights:

- MeOH:H_2O = 4:1 (D475);
- MeOH:H_2O = 1:1 (D478).

The samples obtained were analyzed according to the internal specification corresponding to the pharmacopoeial monograph (**Table 2**).

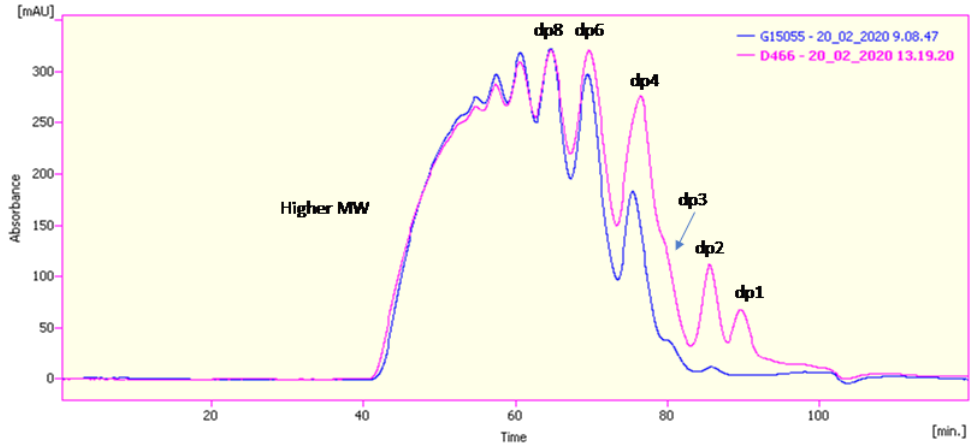
To quantify the effect of the solvent ratio on the composite product, enoxaparin sodium was analyzed by the method of two-dimensional NMR spectroscopy (HSQC) (**Table 3**). In particular, the analysis of the distribution of oligosaccharide fractions was carried out by the SEC method (**Figure 1**).

According to **Table 2**, sample D475 does not meet the requirements of the specification by the "Identification" indicator.

According to a more detailed 2D-NMR analysis, both samples (D475 and D478) represent a high degree of depolymerization, which is evidenced by the low values of normal reduced ANSaRed, MNSaRed residues and high values of 1,6-anhydro ANS/MNS, respectively (**Table 3**). This is the result of the technological parameters of the depolymerization reaction, which obviously needed to be corrected.

Table 2. The results of the analysis of samples of purified enoxaparin sodium with varying solvent ratios according to the specification of JSC Farmak

Parameter	Requirements	D475	D478
Description	A white or almost white powder or crystals	meets	meets
Solubility	Very soluble in water	meets	meets
Loss on drying, %	Not more than 10.0%	8.59	7.52
pH	6.2–7.7	6.31	8.09
Sodium	11.3–13.5	12.4	13.2
Specific absorption	14.0–20.0	18.5	17.1
Residual amounts of organic solvents, ppm	methanol – not more than 0.3% (3000 ppm)	343	13421
	methylene chloride – not more than 0.06% (600 ppm)	0	0
Nitrogen, %	1.5–2.5	1.8	1.8
Molar ratio of sulfate ions to carboxylate ions	not less than 1.8	2.92	5.38
Quantitative analysis	factor Xa activity 90 EU – 125 EU	107.9	104.5
	factor IIa activity 20.0 EU – 35.0 EU	28.1	27.8
	factors Xa/IIa activity ratio 3.3–5.3	3.8	3.8
Identification (the average relative molecular weight and molecular weight distribution)	3800–5000 Da	3978	4239
	<2000 Da 12.0–20.0%	21.9	17.8
	2000–8000 Da 68.0–82.0%	71.1	73.7
Identification (the content of 1,6-anhydro derivatives)	15–25%	23.3	21.8



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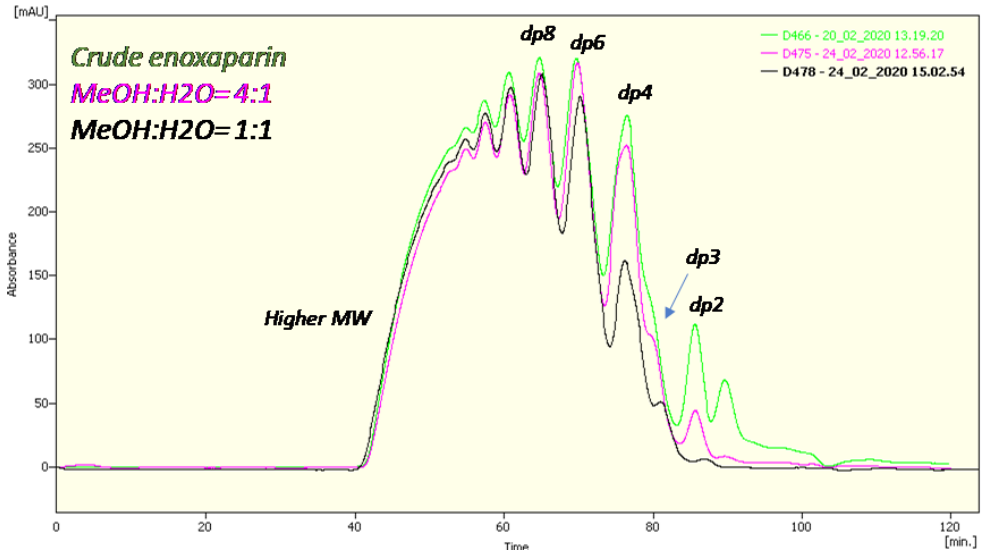

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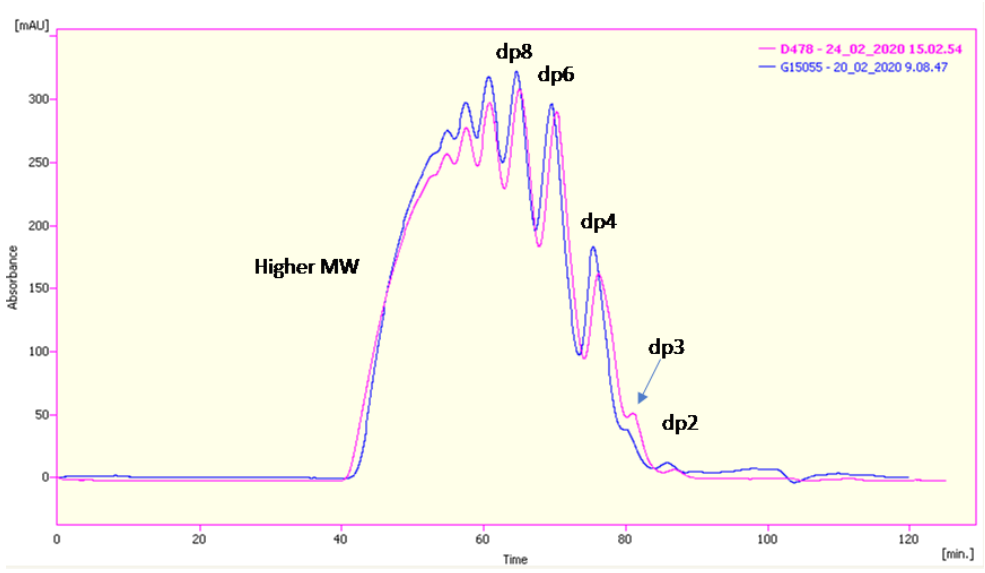

c

Figure 1. Distribution profiles of oligosaccharide fractions of treated samples of enoxaparin compared to Clexane®: (**a**) distribution of oligosaccharide fractions of Clexane® (blue) and crude enoxaparin D466 (pink); (**b**) distribution of oligosaccharide fractions of crude enoxaparin (green), purified enoxaparin D475 (pink) and D478 (black); (**c**) distribution of oligosaccharide fractions of Clexane® (blue) and purified enoxaparin D478 (pink)

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Table 3. The results of the analysis of samples of purified enoxaparin sodium with varying amounts of methanol for precipitation by the HSQC method (2D-NMR)

	Crude enoxaparin sodium	MeOH:H_2O 4:1	MeOH:H_2O 1:1	Clexane	
Amines	D466	D475	D478	min	max
ANS,6xaRed	8.4	7.5	7.4	7.8	9.0
ANS,6XbRed	0.9	1.2	0.8	1.0	1.2
ANAc,6xaRed	0.4	0.4	0.4	0.3	0.4
1,6anANS	3.3	3.3	3.0	2.0	2.3
1,6anMNS	3.9	3.5	3.2	2.4	2.5
MNS,6XaRed	2.4	2.2	2.0	2.6	3.0
%A6S	78.7	80.2	80.8	81.8	82.9
Uronic acid					
ΔU42S	20.4	19.0	17.5	17.3	18.1
ΔU4	1.9	1.7	1.5	1.1	1.2
Epox	1.3	1.4	1.4	0.2	0.6
GalA	1.8	1.9	1.9	1.2	1.8
ΔU42S/ΔU	10.7	11.3	11.5	15.7	15.1

Notes:

Abbreviation	Stands for
ANS,6X-αRed	reducing *N*-sulfated-α-*D*-glucosamine
ANS,6XβRed	reducing *N*-sulfated-β-*D*-glucosamine
ANAc,6X-αRed	reducing *N*-acetyl α-*D*-glucosamine
1,6anANS	2-amino-1,6-anhydro-2-deoxy-β-*D*-glucopyranose
1,6anMNS	2-amino-1,6-anhydro-2-deoxy-β-*D*-mannopyranose
MNS,6XαRed	reducing *N*-sulfated-α-*D*-mannosamine
%A6S	*N*-sulfated/acetylated 6-*O*-sulfated α-*D*-glucosamine/mannosamine percent
ΔU42S	2-*O*-sulfo-4-deoxy-α-*L*-threo-hex-4-enopyranosil uronic acid
ΔU4	4-deoxy-α-*L*-threo-hex-4-enopyranosil uronic acid
epox	epoxide residue
GalA	galacturonic acid

The profiles of the distribution of fractions obtained in the samples synthesized demonstrate the dynamics of the distribution of the molecular weight depending on the type of the sample. Thus, crude enoxaparin sodium (D466, **Figure 1a**) coincides with the profile of the originator in the area of high-molecular fragments, while low-molecular residues remain overestimated compared to Clexane®. Reprecipitation with the use of the solvent ratio of MeOH:H_2O = 4:1 (D475, **Figure 1b**) did not give the expected result in reducing low-molecular-weight particles, but the ratio of MeOH:H_2O = 1:1 (D478, **Figure 1c**), on the contrary, showed a significant effect in this area, making this sample as close as possible to the originator.

According to the analyses conducted, the MeOH:H_2O 4:1 ratio option can be immediately excluded from the study, while the 1:1 solvent ratio experiment was repeated after adjusting the parameters of the chemical ß-elimination stage to create a kind of the correct molecular framework.

2. According to the results of the experiment on setting the technological parameters of the depolymerization stage, the optimal parameters of the process were determined:

- the ratio of "alkali/benzyl ester of heparin" – 0.06;
- reaction temperature – 57°C;
- the reaction time – an interval of 1.5–2 hours.

Samples of crude enoxaparin obtained according to these parameters were reprecipitated with the solvent ratio of:

- MeOH:H_2O = 2:1 (D492 and D494);
- MeOH:H_2O = 1:1 (D493 and D495).

The samples obtained were also analyzed according to the specification developed based on the pharmacopoeial monograph. The results and comparison of sample indicators are given in **Table 4**. For these samples, the compositional analysis by the HSQC method (**Table 5**) and the molecular weight distribution by the SEC method (**Figure 2**) were also determined.

The samples of purified enoxaparin obtained were analyzed according to the specifications of JSC Farmak. These samples, as expected, demonstrated compliance with the regulated requirements of the monograph in terms of "Identification" (the average relative molecular weight and molecular weight

Table 4. The results of the analysis of samples of purified enoxaparin sodium with a change in the ratio of solvents according to the specification of JSC Farmak

Parameter	Requirements	MeOH:H_2O 2:1		MeOH:H_2O 1:1	
		D492	D494	D493	D495
Description	A white or almost white powder or crystals	meets	meets	meets	meets
Solubility	Very soluble in water	meets	meets	meets	meets
Loss on drying, %	Not more than 10.0%	6.13	8.71	7.30	6.76
pH	6.2–7.7	7.72	7.23	6.93	7.06
Sodium	11.3–13.5	11.3	11.7	11.8	11.6
Specific absorption	14.0–20.0	17.3	17.1	15.2	15.5
Residual amounts of organic solvents, ppm	methanol – not more than 0.3% (3000 ppm)	583	150	1110	5407
	methylene chloride – not more than 0.06% (600 ppm)	0	0	0	0
Nitrogen, %	1.5–2.5	1.9	2.0	1.9	2.0
Molar ratio of sulfate ions to carboxylate ions	not less than 1.8	2.4	3.5	2.3	2.9
Quantitative analysis	factor Xa activity 90 EU – 125 EU	109.1	101.6	113.7	103.6
	factor IIa activity 20,0 EU – 35,0 EU	29.7	29.7	36.1	35.0
	factors Xa/IIa activity ratio 3.3–5.3	3.7	3.4	3.1	3.0
Identification (the average relative molecular weight and molecular weight distribution)	3800–5000 Da	4553	4625	4880	4905
	< 2000 Da 12.0%–20.0%	18.1	17.0	12.5	12.5
	2000-8000 Da 68.0%–82.0%	70.2	71.0	74.5	74.2
Identification (the content of 1,6-anhydroderivatives)	15–25%	17.2	19.9	15.6	18.7

distribution). According to the results of the compositional analysis, we observe a tendency to decrease the number of residues at the reducing ends of the molecule – ANS/MNSred, 1,6anMNS/ANS and structures at the non-reducing ends of the molecule – ΔU42S, ΔU4, which, however, still does not coincide with the variation ranges of Clexane®.

The analysis of the molecular weight distribution shows that the samples D492 and D494 obtained with the ratio of MeOH:H_2O=2:1 have a larger number of residues with a low-molecular weight than Clexane®. Similarly, high-molecular-weight fragments are more common in D492 and D494 than in Clexane® (**Figure 2**).

Table 5. The results of the analysis of samples of purified enoxaparin sodium with varying amounts of methanol for precipitation by the HSQC method (2D-NMR)

	Crude enoxaparin		MeOH:H_2O 2:1		MeOH:H_2O 1:1		Clexane	
	1.5 h	2 h						
Amines	D484	D485	D492	D494	D493	D495	min	max
ANS,6xaRed	10.1	9.6	9.8	9.0	8.5	8.0	7.8	9.0
ANS,6XbRed	1.3	1.2	1.0	1.2	1.2	1.1	1.0	1.2
ANAc,6xaRed	0.6	0.6	0.5	0.5	0.4	0.4	0.3	0.4
1,6anANS	2.2	2.5	2.0	2.1	1.7	1.9	2.0	2.3
1,6anMNS	2.5	2.7	2.2	2.3	1.9	2.1	2.4	2.5
MNS,6XaRed	2.9	2.7	2.7	2.4	2.4	2.2	2.6	3.0
%A6S	80.4	80.0	80.8	80.3	81.8	81.4	81.8	82.9
Uronic acid								
ΔU42S	19.2	19.2	18.1	17.6	15.7	15.7	17.3	18.1
ΔU4	1.7	1.7	1.5	1.5	1.2	1.2	1.1	1.2
Epox	0.9	0.9	0.9	1.0	0.9	1.0	0.2	0.6
GalA	2.0	1.9	1.9	1.9	1.9	1.8	1.2	1.8
ΔU42S / ΔU	11.3	11.1	11.9	11.8	13.3	12.7	15.7	15.1

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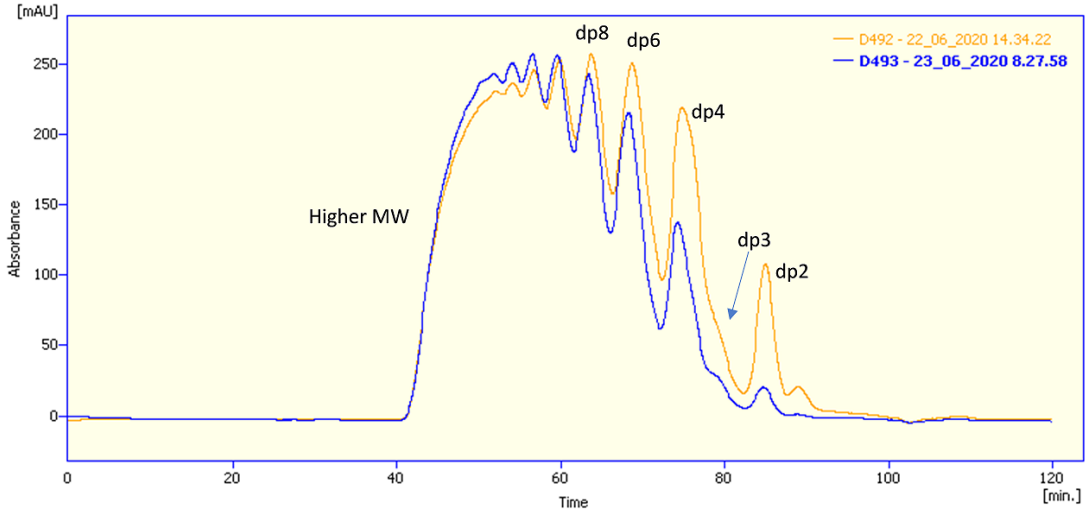

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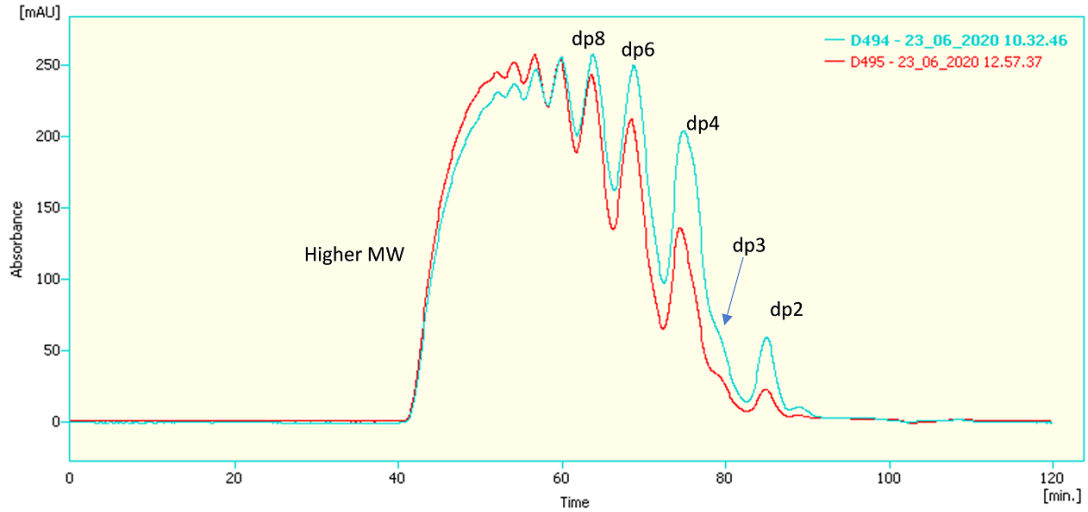

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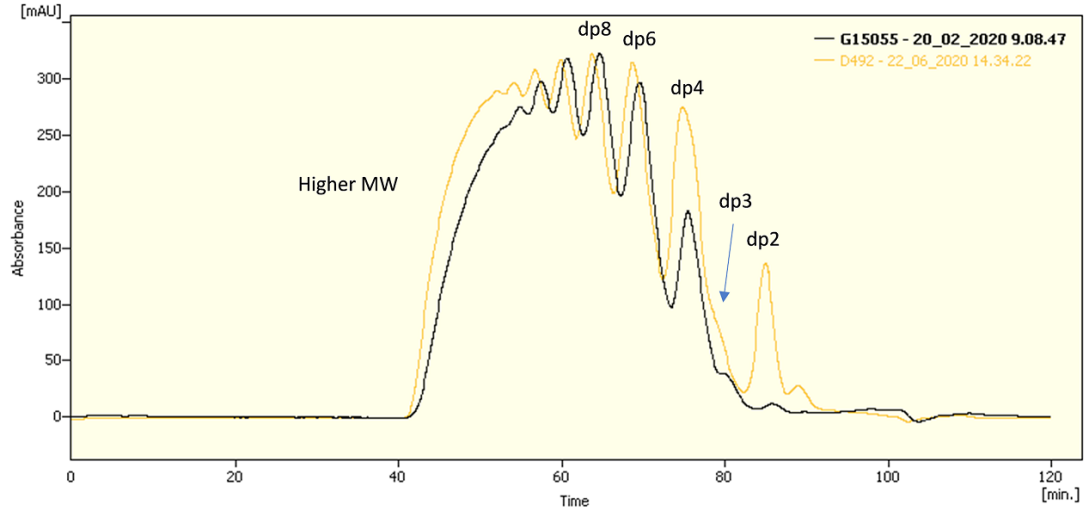
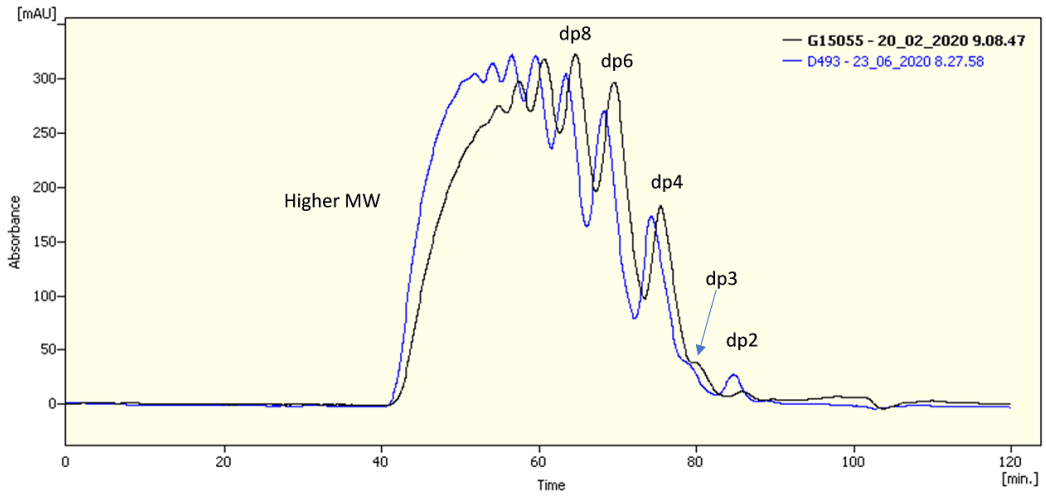


f

Figure 2. Distribution profiles of oligosaccharide fractions of treated samples of purified enoxaparin compared to Clexane®: (**d**) distribution of oligosaccharide fractions of purified enoxaparin D492 (yellow) and D493 (blue); (**e**) distribution of oligosaccharide fractions of purified enoxaparin D494 (blue) and D495 (red); (**f**) distribution of oligosaccharide fractions of Clexane® (black) and D492 (yellow); (**g**) distribution of oligosaccharide fractions of Clexane® (black) and D493 (blue); (**h**) distribution of oligosaccharide fractions of Clexane® (black) and D494 (blue); (**i**) distribution of oligosaccharide fractions Clexane® (black) and D495 (red) (see on the next page)



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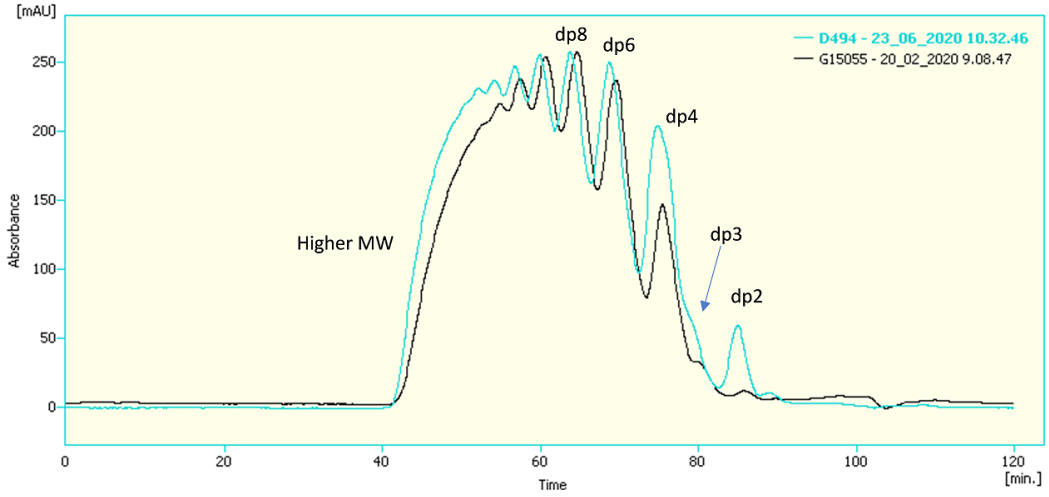

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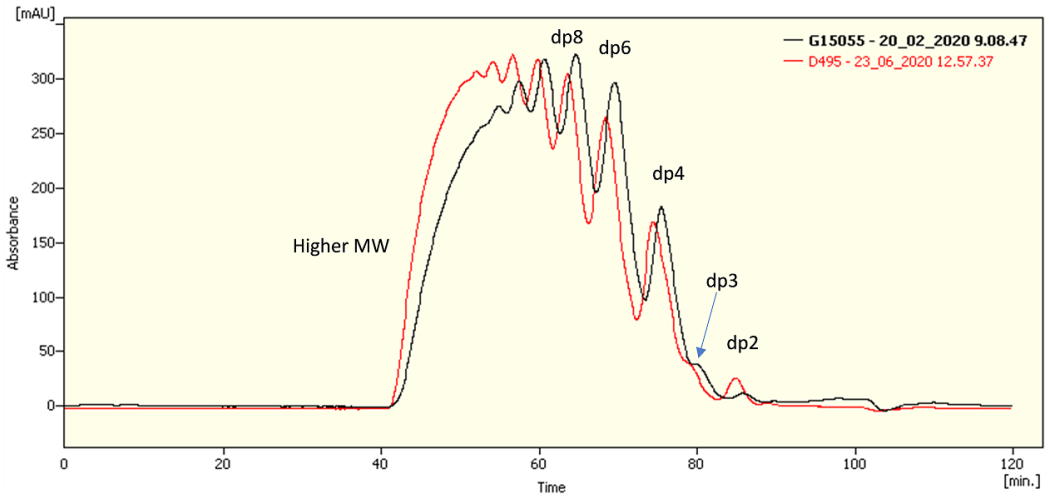

i

Figure 2. Distribution profiles of oligosaccharide fractions of treated samples of purified enoxaparin compared to Clexane®: (**d**) distribution of oligosaccharide fractions of purified enoxaparin D492 (yellow) and D493 (blue); (**e**) distribution of oligosaccharide fractions of purified enoxaparin D494 (blue) and D495 (red); (**f**) distribution of oligosaccharide fractions of Clexane® (black) and D492 (yellow); (**g**) distribution of oligosaccharide fractions of Clexane® (black) and D493 (blue); (**h**) distribution of oligosaccharide fractions of Clexane® (black) and D494 (blue); (**i**) distribution of oligosaccharide fractions Clexane® (black) and D495 (red)

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Table 6. Calculation of the E-factor of the purification stage of crude enoxaparin sodium considering the solvent regeneration

Materials	Quantity of materials, kg		Product yield, kg	E-factor	E-factor that takes into account the regeneration
	Without the solvent regeneration	With the solvent regeneration			
Crude enoxaparin sodium	0.1	0.1	0.08	14	5.25
Sodium chloride	0.1	0.1			
Methanol	1.0	0.3			
	1.2	0.5			

Samples D493 and D495 (**Figures 2g,i**) show a profile similar to Clexane® in the range of short oligomers, indicating that the ratio of MeOH:H_2O=1:1 allows better control of the number of low molecular weight oligomers. However, the intensity of the high-molecular range is higher than that of Clexane®. A decrease in the number of short particles, depending on the amount of methanol, shifts the molecular weight distribution towards high-molecular weight.

The methanol regeneration was envisaged as the greening stage of the synthesis. The regeneration yield was 70%. The calculation of the E-factor considering the regeneration is shown in **Table 6**. The E-factor value obtained without the methanol regeneration is 14. Recalculation of the E-factor considering the methanol regeneration is 5.25.

■ Conclusions

In this experimental study, it was possible to clearly demonstrate the effect of the ratio of solvents at the stage of purification of crude enoxaparin on the number and distribution of oligosaccharide fractions in the molecule. Thus, it has been found that an increase in the amount of methanol for the reprecipitation of enoxaparin provokes a shift in the profile of the molecular weight distribution towards low molecular weight oligosaccharides, respectively, a smaller amount of methanol allows obtaining the profile closest to the originator in the area of low-molecular-weight residues. The ratio of MeOH:H_2O = 1:1 makes it possible to obtain samples that are better comparable in terms of the composition to the ranges of Clexane®, except for the terminal residues. However, during the study, it was found that with the reduction of low-molecular-weight residues, the intensity in the area of high-molecular-weight oligosaccharides increased. Summarizing the results obtained, it can be concluded that the ratio of MeOH:H_2O = 1:1 is acceptable for obtaining a substance close to the original one. As an indicator of the effectiveness of the method of the purification stage of enoxaparin sodium in view of the principles of "green" chemistry, there is an E-factor reduced from 14 to 5.25 by the methanol regeneration.

■ Experimental part

This study was conducted during 2019–2021.

The treated samples of purified enoxaparin sodium were analyzed according to the internal specification developed based on the pharmacopoeial monograph. For detailed structural characterization of enoxaparin sodium samples obtained under different conditions, the analysis was performed by specialists of the Ronzoni Institute (Italy) using the methods of 2D-NMR (heteronuclear single quantum coherence spectroscopy) and size exclusion chromatography (SEC). The results of the analysis were compared with the results of the analysis of the original Clexane® referring to the database formed by the Ronzoni Institute.

Clexane® from Sanofi-Aventis was obtained from commercial suppliers.

All samples were analyzed before the expiration date.

The pH test was determined on a Mettler Toledo Seven compact S220 pH meter (Switzerland) (*Ph. Eur. 2.2.3*), the analysis of loss on drying was performed on a Pol-Eko Aparatura slw 53 (*Ph. Eur. 2.2.32*); nitrogen was analyzed on a Vapodest VAP 30s Gerhardt GmbH Distillation System (*Ph. Eur. 2.5.9*); the analysis of residual amounts of organic solvents was carried out by the head-space gas chromatography method on an Agilent GC 7890B chromatograph (USA), column DB-624, 60m×0.32mm, with a layer thickness of 1.8 µm (*Ph. Eur. 2.2.28, 2.2.46*); specific absorption was measured on a Mettler Toledo UV-5 spectrophotometer (*Ph. Eur. 2.2.25*); identification (the average relative molecular weight and

molecular weight distribution) was performed on a Shimadzu chromatograph (Japan), column X_TSKgel G2000SW (300mm×7.8mm×5μm) with a Viscotec 305 detector, Malvern Instruments LTD (England) (*Ph. Eur. 2.2.30*). The content of 1,6-anhydro derivatives was measured by the LC method on a Metrohm chromatograph (*Ph. Eur. 2.2.29*). The molar ratio of sulfate ions to carboxylate ions was measured on a Seven Compact S230 conductometer (Switzerland) (*Ph. Eur. 2.2.38*).

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Information about the authors:
Yuliia V. Bovsunovska (*corresponding author*), Ph.D. student of the Pharmaceutical Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; Engineer-technologist, JSC Farmak; https://orcid.org/0000-0001-6525-5520; e-mail for correspondence: juliabovsu@gmail.com.
Vitalii V. Rudiuk, Head of the API Synthesis Laboratory, JSC Farmak; https://orcid.org/0000-0003-3440-1139.
Nataliia V. Harna, Ph.D. in Pharmacy, Associate Professor of the Pharmaceutical Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0003-2918-4985.
Olha S. Holovchenko, Ph.D. in Pharmacy, Associate Professor of the Pharmaceutical Chemistry Department, National University of Pharmacy of the Ministry of Health of Ukraine; https://orcid.org/0000-0002-5252-5517.
Victoriya A. Georgiyants, D.Sc. in Pharmacy, Professor, Head of the Department of Pharmaceutical Chemistry, National University of Pharmacy of the Ministry of Health of Ukraine; http://orcid.org/0000-0001-8794-8010.