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Analytical Support For The Pharmaceutical Development Of An Original Combined Hepatoprotective Drug In Tablet Form

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hepatoprotective drug; liquid chromatography; pharmaceutical development; selenomethionine; silymarin; tablets; thioctic acid; UV spectrophotometry;validation; pharmaceuticals analysis

ABSTRACT

Analytical support for the pharmaceutical development of key quality indicators of an original combined drug containing thioctic acid, silymarin, and selenomethionine, in accordance with the requirements of the State Pharmacopoeia of Ukraine (harmonized with the European Pharmacopoeia, Eur.Ph.) for oral dosage forms, specifically tablets, was carried out. The methods for the identification and quantitative determination of thioctic acid in the finished dosage form using liquid chromatography were developed, standardized, and validated. Similarly, methods for the identification and quantitative determination of silymarin using UV spectrophotometry were developed, standardized, and validated. Validation studies for the developed quantitative methods for thioctic acid and silymarin demonstrated that the validation characteristics, such as the predicted total uncertainty of the analysis, specificity, linearity, precision (repeatability), and accuracy, were

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found to meet the established acceptance criteria. A method for the identification of selenomethionine using a color reaction and a method for its quantitative determination using inductively coupled plasma atomic emission spectrometry (ICP-AES) were developed. Finally, the method for the dissolution test of combined tablets containing silymarin using UV spectrophotometry was developed and its suitability was demonstrated.

1. Introduction

Pharmacotherapy of liver diseases remains a major challenge due to the essential functions of the liver and the high prevalence of hepatic disorders, which rank third worldwide after cardiovascular and oncological conditions [1, 2]. These pathologies are associated with multiple etiological factors and often progress to chronic forms, underscoring the need for effective hepatoprotective agents. The primary pharmacological effects of hepatoprotectors include antioxidant, membrane-protective, antitoxic, and mild choleretic activities [3]. Combination therapy with agents of diverse mechanisms, particularly antioxidants, is considered most effective [4–6].

The rationale for the selected components is based on their complementary properties. Milk thistle (Silybum marianum) extract (silymarin) demonstrates pronounced antioxidant, membrane-protective, and metabolic effects, with silybin identified as the most active constituent [7–12]. Alpha-lipoic acid (thioctic acid) is a naturally occurring compound with broad antioxidant activity, capable of regenerating glutathione, ascorbate, and tocopherol, while also exerting detoxifying, hypoglycemic, hypolipidemic, immunotropic, and neuroprotective effects [13, 14]. Selenium, an essential trace element, is a cofactor of glutathione peroxidases and other selenoproteins; its deficiency is associated with chronic hepatitis, highlighting its importance in antioxidant defence [15].

Taken together, silymarin, alpha-lipoic acid, and selenium provide interdependent and complementary hepatoprotective effects. Their combined use targets free radical–induced membrane damage, suppresses inflammatory-necrotic responses, and may inhibit fibrosis progression and reduce malignant transformation risk. Therefore, the development of an original combined hepatoprotective tablet containing thioctic acid, silymarin, and selenomethionine is a promising approach for the treatment of liver diseases.

The aim of this study is to provide analytical support for the pharmaceutical development and to standardize quality control methods for an original combined drug containing α-lipoic acid, silymarin, and selenomethionine, based on key indicators and in compliance with the requirements of the State Pharmacopoeia of Ukraine (harmonized with the European Pharmacopoeia) for oral dosage forms, specifically tablets.

2. Materials and Methods

The study includes active pharmaceutical ingredients (APIs) — α-lipoic acid (5-[(3RS)-1,2-Dithiolan-3-yl]pentanoic acid), silymarin, selenomethionine ((S)-2-amino-4-(methylselenyl)butyric acid), and a pharmaceutical formulation in tablet form containing these APIs. The formulation consisted of film-coated tablets, light brownish-yellow to yellow-brown in color, elongated in shape, with convex upper and lower surfaces and a score line for splitting.

The α-lipoic acid substance is described in the Eu-

ropean Pharmacopoeia 11th Edition (Eur.Ph. 11.0) monograph "Thioctic acid" [16]. *Appearance*: yellow crystalline powder; *Solubility*: very slightly soluble in water, very soluble in dimethylformamide, freely soluble in methanol. For the development of the formulation, α-lipoic acid produced by Shanghai Modern Pharmaceutical Co., Ltd., China, was used. The quality specifications of this manufacturer comply with the requirements of Eur.Ph. 11.0.

Silymarin (produced by IVAX, Czech Republic) is a natural mixture of isomeric flavonolignans, primarily consisting of six components: silybin A, silybin B, isosilybin A, isosilybin B, silychristin, and silydianin A. According to the manufacturer's specifications, the silymarin substance is an amorphous, brownish-yellow powder, hygroscopic, practically insoluble in *water Ph.Eur*., soluble *in ethyl acetate Ph.Eur*., *methanol Ph.Eur*., and *96% ethanol Ph.Eur*. The silymarin substance is not described in any pharmacopoeia worldwide.

Selenomethionine is described in the current USP–NF monograph "Selenomethionine" (USP 46–NF 41, online) [17]. The substance appears as a white or almost white crystalline powder and is soluble in hot water and very slightly soluble in 96 % ethanol and methanol. For the development of the formulation, Selenomethionine produced by SABINSA EUROPE GmbH (Germany) was used. The quality specifications of this manufacturer comply with the requirements of USP–NF.

The following standards were used: thioctic acid EP CRS, silymarin reference standard (IVAX, Czech Republic), and selenium (IV) solution reference standard (State Pharmacopoeial Center of Ukraine, 022.57-96).

The analysis was carried out using liquid chromatography (LC) for thioctic acid, ultraviolet (UV) absorption spectrophotometry for silymarin, and inductively coupled plasma atomic emission spectrometry (ICP-AES) for selenium, following the relevant European Pharmacopoeia methods (Eur.Ph., 2.2.29, 2.2.46, 2.2.25, 2.2.22).

Development of the composition and manufacturing technology of the drug

At the initial stage of pharmaceutical development, the selection and justification of the optimal amounts of active substances were carried out. The chosen dosages of the active ingredients correspond to their dosages in the respective monocomponent preparations (Legalon 140 capsules, containing 140 mg of silymarin per capsule ("Madaus GmbH," Germany), and Berlition 300 oral film-coated tablets, containing 300 mg of thioctic acid per tablet ("Berlin-Chemie," Germany)). In this context, particular attention was paid to the analysis of the physicochemical and pharmaco-technological properties of the active substances, their quality specifications, and their compatibility with excipients in the dosage form.

The study of the physicochemical and pharmaco-technological properties of the active ingredients revealed differences in their technological characteristics: thioctic acid exhibited good compressibility, whereas selenomethionine and silymarin showed low compressibility but had nearly twice the bulk properties of thioctic acid and better flowability. It was also found that the mixture of thioctic acid, selenomethionine, and silymarin exhibited inadequate flowability and relatively low compressibility. Therefore, in the development of the tablet formulation, excipients that improve the flowability and compressibility of the tablet mass should be used to ensure the required technological characteristics of the mass for tableting.

To improve the compressibility of the tablet mass, microcrystalline cellulose, which exhibits high compressibility, was used. Sodium starch glycolate and croscarmellose sodium were employed as disintegrants. Croscarmellose sodium was added to maintain tablet strength and enhance disintegration and was incorporated during the dusting process. Mannitol was used as an inert filler/diluent. Copovidone (Plasdone S-630) was included as a binder. Potato starch was introduced during the dusting step as a disintegrant to enhance the hydrophilicity of the tablets. Macrogol was added as a solubilizer and, during tableting, partially acted as a lubricant, reducing friction at contact points and facilitating particle deformation due to the adsorptive decrease

in particle strength (penetration into microcavities). Magnesium stearate, talc, and Aerosil were used as adhesive, anti-friction, flow-enhancing agents to reduce friction between the tablet mass and the equipment during compression

The wet granulation method was considered optimal for tablet manufacturing, as the direct compression method presented difficulties in distributing the active components evenly in the mixture and maintaining homogeneity during tableting. Tablets obtained by direct compression did not meet the State Pharmacopoeia of Ukraine (SPhU) requirements for friability and mass uniformity. Based on the study of the pharmaco-technological properties of the granulated mixtures and analysis of the quality of the resulting tablets, a 5% aqueous solution of copovidone was selected as the granulating agent. A process of separate granulation of the tablet masses was proposed, as the active pharmaceutical ingredients possess different physicochemical properties: selenomethionine is soluble in hot water, whereas thioctic acid and silymarin are practically insoluble in water.

Since the dosage of selenomethionine in the drug is 0.008% of the tablet mass, it was incorporated into the tablet mass by preparing a trituration with mannitol. The trituration of selenomethionine and mannitol was dissolved in purified water and added to the aqueous solution of copovidone. The resulting granulating agent A was used to produce the thioctic acid granulate. For the preparation of the silymarin granulate, granulating agent B was used, which was prepared by dissolving the trituration of selenomethionine and mannitol in purified water with the addition of macrogol, followed by incorporation into the aqueous copovidone solution. The resulting granulates were then subjected to drying. The proper selection of the drying temperature regime is crucial for obtaining a high-quality product. Granulate 1 was dried in a tray dryer at 35 ± 5 °C for 10–12 hours, as thioctic acid melts above 60 °C. Granulate 2 was dried at 45 ± 5 °C for 10–12 hours.

Since the dosage of selenomethionine in the formulation is 0.008% of the tablet mass, it was incorporated into the tablet mass by preparing a trituration with mannitol. The trituration of selenomethionine and mannitol was dissolved in purified water and added to the aqueous solution of copovidone. The resulting granulating agent A was used to prepare the thioctic acid granulate. For the preparation of the silymarin granulate, granulating agent B was employed, which was prepared by dissolving the trituration of selenomethionine and mannitol in purified water with the addition of macrogol and subsequent incorporation into the aqueous copovidone solution. The obtained granulates were then subjected to drying. The correct selection of the drying temperature regime is critical for obtaining a high-quality product. Granulate 1 was dried in a tray dryer at 35 ± 5 °C for 10–12 hours, as thioctic acid melts above 60 °C. Granulate 2 was dried at 45 ± 5 °C for 10–12 hours.

Dry granulation was performed by mixing granulates 1 and 2, followed by dusting with croscarmellose sodium, potato starch, Aerosil, talc, and magnesium stearate. After tableting, the tablets were film-coated with Opadry II Yellow to mask the unpleasant taste (bitter taste of thioctic acid and silymarin), to protect the tablets from external factors, and to improve their appearance and ease of use.

Thus, as a result of the studies on the properties of the active pharmaceutical ingredients and excipients according to various criteria, the optimal composition of the drug was selected, a rational technology for preparing the tablet mass using the wet granulation method was developed, the composition of the granulating agent was determined, and the critical drying temperature for the granulates was established.

Identification.

Thioctic acid. Liquid chromatography (Eur.Ph., 2.2.29). The retention time of the principal peak in the chromatogram of the *test solution* during the quantitative determination of thioctic acid should correspond to the retention time of the principal peak in the chromatogram of the *reference solution*.

Silymarin. The ultraviolet absorption spectrum of the *test solution*, obtained during the quantitative determination of silymarin, in the range of 240 nm to 350 nm should exhibit a maximum absorption at a

wavelength of (288 ± 2) nm.

Selenomethionine. Five grams of powdered tablets are placed into a 100 ml flask, 50 ml of *Water R* is added, the mixture is shaken for 5 minutes, and then centrifuged at 8000 rpm for 10 minutes. One milliliter of the resulting filtrate is placed into a 15 ml evaporating dish, 1.0 ml of 0.2% *ninhydrin R solution in Water R* is added, and the mixture is evaporated to dryness, resulting in a purple coloration

Assay of Thioctic Acid.

Determination was performed using liquid chromatography according to the European Pharmacopoeia (Eur. Ph., 2.2.29). All solutions were protected from light.

Solvent mixture: an equal-volume mixture of *acetonitrile R1* and a 0.7 g/L solution of *potassium dihydrogen phosphate R*, previously adjusted to pH 3.6 *with phosphoric acid R*.

*Test Solution.*Approximately 0.13 g (accurately weighed) of powdered tablets was placed in a 50 mL volumetric flask, 40 mL of the solvent mixture was added, and the mixture was shaken for 15 minutes. The volume was then adjusted to the mark with the same solvent, mixed thoroughly, and filtered through a "blue ribbon" filter, discarding the first 2 mL of filtrate.

Reference Solution. Dissolve 50.0 mg of thioctic acid CRS in the solvent mixture and dilute to 50.0 mL with the same solvent mixture.

Chromatography was performed on a liquid chromatograph equipped with a UV detector under the following conditions:

Column: Waters Spherisorb ODS2

Dimensions: l = 0.25 m, Ø = 4.6 mm

Stationary phase: octadecylsilyl silica gel for chromatography R (5 μm)

Temperature: 35 °C

Mobile phase: a mixture of 8 volumes of acetonitrile R1, 41 volumes of a 0.7 g/L solution of potassium dihydrogen phosphate R previously adjusted to pH 3.0 with phosphoric acid R, and 51 volumes of methanol R

Flow rate: 1.2 mL/min

Detection: UV spectrophotometry at 215 nm

Injection volume: 20 μL

System suitability:

Column efficiency, calculated from the thioctic acid peak in the reference solution chromatogram, should be not less than 5000 theoretical plates

Symmetry factor: maximum 1.8 for the thioctic acid peak in the reference solution chromatogram

Relative standard deviation, calculated for the thioctic acid peak area in three consecutive *reference solution* chromatograms, should be not more than 2.0%

Limits:

The content of thioctic acid (X_1) in a single tablet, expressed in grams, is calculated using the following formula:

$$X_1 = \frac{S_1 * m_0 * 50 * P * b}{S_0 * 50 * m_1 * 100} = \frac{S_1 * m_0 * P * b}{S_0 * m_1 * 100},$$

Where: S1 - mean peak area of thioctic acid obtained from the chromatograms of the test solution;

S0 - mean peak area of thioctic acid obtained from the chromatograms of the reference solution;

m0 - mass of thioctic acid CRS used, in grams;

m1 - mass of the sample (tablet powder) used, in grams;

P - thioctic acid content in thioctic acid CRS, expressed as a percentage;

b - average weight of a single tablet, in grams.

The content of $\boldsymbol{C_8H_{14}O_2S_2}$ *(thioctic acid)* in a single tablet should be between 0.285 g and 0.315 g, calculated based on the average tablet weight.

Assay of Silymarin

Determination was performed using ultraviolet (UV) absorption spectrophotometry according to the European Pharmacopoeia (Eur. Ph., 2.2.25).

Test Solution. Approximately 0.15 g (accurately

weighed) of powdered tablets was placed in a 50 mL volumetric flask, 40 mL of *methanol R* was added, and the mixture was shaken for 10 minutes while heating in a water bath at approximately 50 °C. The solution was then cooled to room temperature, the volume was adjusted to the mark with the same solvent, and filtered through a "blue ribbon" filter, discarding the first 2 mL of filtrate. 1.0 mL of the resulting solution was further diluted with *methanol R* to 50.0 mL.

Reference Solution. Approximately 0.04 g (accurately weighed) of silymarin CRS, previously dried at 100–105 °C to constant weight, was dissolved in *methanol R* while heating in a water bath at approximately 50 °C. The solution was then cooled to room temperature and the volume was adjusted to 50.0 mL with the same solvent. 1.0 mL of the resulting solution was further diluted with *methanol R* to 50.0 mL.

The optical density of the *test solution* was measured using a spectrophotometer at a wavelength of 288 nm in a 10 mm path-length cuvette, using *methanol R* as the blank.

The optical density of the *reference solution* was measured in parallel.

Limits:

The content of silymarin (X_2) in a single tablet, expressed in grams, is calculated using the following formula:

$$X_2 = \frac{A_1 * m_0 * 50 * 50 * P * b}{A_0 * m_1 * 50 * 50 * 100} = \frac{A_1 * m_0 * P * b}{A_0 * m_1 * 100},$$

Where: A1 - optical density of the test solution;

A0 - optical density of the reference solution;

m0 - mass of silymarin CRS used, in grams;

P - silymarin content in silymarin CRS, expressed as a percentage;

m1 - mass of the sample (tablet powder) used, in grams;

b - average weight of a single tablet, in grams.

The content of $\boldsymbol{C_{25}H_{22}O_{10}}$ (silymarin) in a single tablet should be between 0.133 g and 0.147 g, calculated based on the average tablet weight.

Assay of Selenium.

The determination was performed using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to the European Pharmacopoeia (Eur. Ph., 2.2.22).

Reference solution (a): Standard solution containing 1 g/L of selenium (standard reference material of selenium (IV), DSZU 022.57-96).

Reference solution (b): 5.0 mL of solution (a) was placed in a 50 mL volumetric flask, 1 mL of *nitric acid R* was added, and the volume was adjusted to the mark with *water R* and mixed (corresponding to 0.1 g of selenium). The solution was used freshly prepared.

Reference solution (c): 0.1 mL of solution (b) was placed in a 100 mL volumetric flask, 12 mL of *nitric acid R* and 2 mL of 50% *hydrogen peroxide R* were added, the volume was adjusted to the mark with *water R* and mixed (0.1 mg/L of selenium). The solution was used freshly prepared.

Reference solution (d): 0.3 mL of solution (b) was placed in a 100 mL volumetric flask, 12 mL of *nitric acid R* and 2 mL of 50% *hydrogen peroxide R* were added, the volume was adjusted to the mark with *water R* and mixed (0.3 mg/L of selenium). The solution was used freshly prepared.

Reference solution (e): 0.5 mL of solution (b) was placed in a 100 mL volumetric flask, 12 mL of *nitric acid R* and 2 mL of 50% *hydrogen peroxide R* were added, the volume was adjusted to the mark with *water R* and mixed (0.5 mg/L of selenium). The solution was used freshly prepared.

Control solution: 12 mL of *nitric acid R* and 2 mL of 50% *hydrogen peroxide R* were placed in a 100 mL volumetric flask, the volume was adjusted to the mark with *water R* and mixed. The solution was used freshly prepared.

Test solution: Approximately 0.25 g (accurately weighed) of powdered tablets was placed in a 100 mL beaker, moistened with 1–2 mL of *water R*, then 3 mL of *nitric acid R* and 0.5 mL of 50% *hydrogen peroxide R* were added sequentially. The beaker was covered with a watch glass and gently heated for 5–10 minutes until the reaction was complete. After

cooling, 5 mL of *water R* was added, and the watch glass was rinsed over the beaker with 2–3 mL of *water R*. The resulting solution was filtered through a "blue ribbon" filter into a 25 mL volumetric flask, the beaker walls were rinsed three times with small portions (~2 mL) of *water R* through the same filter, and the volume was adjusted to the mark. The solution was used freshly prepared.

Measurement: Measurements were carried out on an ICP-AES spectrometer using the following parameters: selenium analytical line 196.090 nm, plasma power 950 W, solution flow rate 1.85 mL/min. The solutions were nebulized into the plasma sequentially: *reference solutions* (c), (d), (e), and the *test solution*, and the emission intensity at the selenium analytical line was recorded. Between solutions, *water R* was nebulized into the plasma.

The selenium content (C) in the *test solution* was calculated using the spectrometer software.

Limits:

The selenium content (X_3) in a single tablet, expressed in micrograms, is calculated using the following formula:

$$X_3 = \frac{C * 25 * 1000 * b}{1000 * m} = \frac{C * 25 * b}{m},$$

Where:

C - selenium concentration in the test solution, expressed in mg/L;

m – mass of the sample (tablet powder) used, in grams;

b - average weight of a single tablet, in grams..

The content of ***Se*** (*selenium*) in a single tablet should be between 20 μg and 30 μg, calculated based on the average tablet weight.

Dissolution Testing (for Silymarin)

The test was carried out in accordance with the European Pharmacopoeia (Eur. Ph., 2.9.3) using a paddle apparatus (Model Erweka DT6RE). Dissolution medium: 0.5% sodium dodecyl sulfate solution; volume: 900 mL; temperature: 37 ± 0.5 °C; paddle rotation speed: 100 rpm; dissolution time: 45 minutes.

For the test, 1 tablet was placed in the dissolution vessel.

Sodium dodecyl sulfate solution preparation: 5 g of sodium dodecyl sulfate R was placed in a 1 L volumetric flask, dissolved in water R with gentle mixing, the volume was adjusted to the mark with *water R,* and mixed carefully.

Test solution: After 45 minutes, 20 mL was withdrawn from the center of the dissolution vessel and filtered through a "blue ribbon" filter, discarding the first 2 mL of filtrate. 5.0 mL of the resulting solution was diluted with 0.5% sodium dodecyl sulfate solution to 50.0 mL.

Reference solution: Approximately 0.05 g of silymarin CRS, previously dried at 100–105 °C to constant weight, was placed in a 250 mL volumetric flask, 200 mL of 0.5% sodium dodecyl sulfate solution was added, and the mixture was treated in an ultrasonic bath at approximately 40 °C for 20 minutes. The solution was then cooled to room temperature, the volume was adjusted to the mark with the same solvent, and filtered through a "blue ribbon" filter, discarding the first 2 mL of filtrate. 5.0 mL of the resulting solution was diluted with 0.5% sodium dodecyl sulfate solution to 50.0 mL.

The optical density of the *test solution* was measured using a spectrophotometer at a wavelength of 288 nm in a 10 mm path-length cuvette, using 0.5% sodium dodecyl sulfate solution as the blank.

The optical density of the *reference solution* was measured in parallel.

Limits:

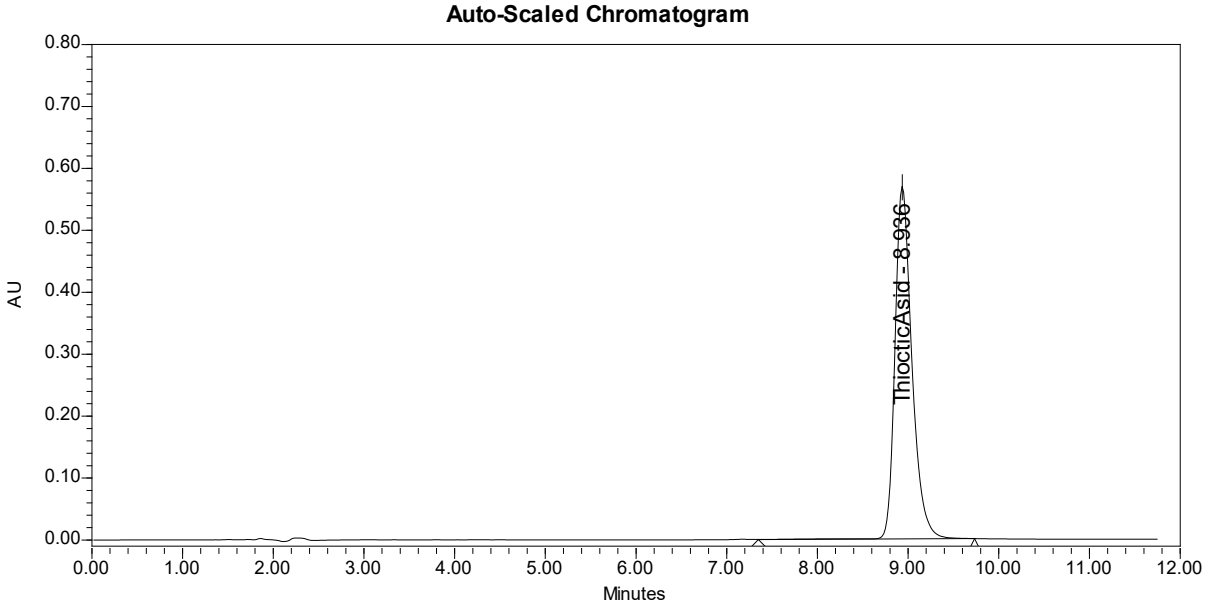
The amount of silymarin (X_1) released from the tablet into the dissolution medium, expressed as a percentage, is calculated using the following;

$$\text{formula: } X_1 = \frac{A_1 * m_0 * 5 * 900 * 50 * P * 100}{A_0 * 1 * 250 * 50 * 5 * 100 * 0,14} = \frac{A_1 * m_0 * P * 3,6}{A_0 * 0,14},$$

Where: A1 - optical density of the test solution;

A0 - optical density of the reference solution;

m0 - mass of silymarin CRS used, in



Integration Results

	Name	RT	Start Time	End Time	Area	Height	% Area	Int Type
1	ThiocticAsid	8.936	7.350	9.733	7351303	569193	100.00	VB

Figure 1. *Chromatogram of the test solution for the determination of thioctic acid*

grams;

P - silymarin content in silymarin CRS, expressed as silibinin equivalent, in percentage;

0.14 - silymarin content specified in the section "Composition per tablet," in grams.

Corresponding to 45 minutes:

Stage 1: Each of the 6 tablets releases not less than 80% of silymarin, based on the content specified in the "Composition per tablet" section.

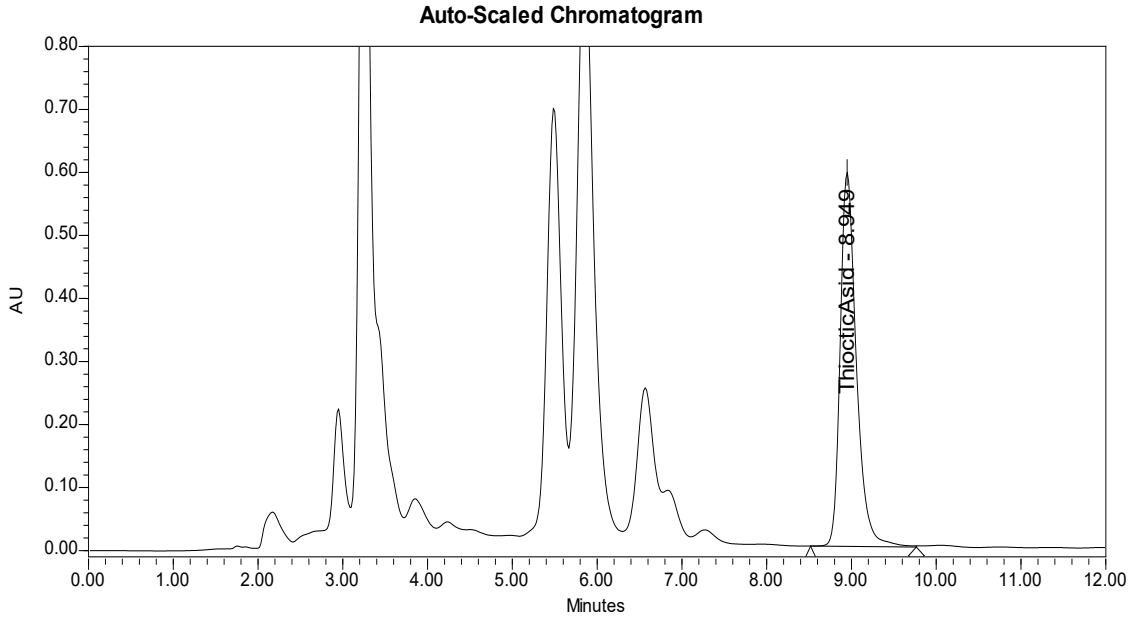
Stage 2: The average release from 12 tablets is ≥ 80%, and no tablet releases less than 60% of silymarin based on the content specified in the "Composition per tablet" section.

Stage 3: The average release from 24 tablets is ≥ 80%. No more than 2 tablets release less than 60%, and no tablet releases less than 50% of silymarin based on the content specified in the "Composition per tablet" section.

The main qualitative and quantitative attributes of the drug are Identification, Assay, and Dissolution, the development of which is the focus of this study. In addition, the drug was standardized according to all quality tests required for oral dosage forms, specifically tablets, in accordance with the European Pharmacopoeia[16]: Uniformity of mass (Eur. Ph., 2.9.5), Uniformity of dosage units (Eur. Ph., 2.9.40), and Disintegration (Eur. Ph., 2.9.1, Test A).

3. Results and Discussion

For the identification of thioctic acid in the drug, a liquid chromatography (LC) method was proposed, which also allows the quantitative determination of this substance in the formulation. The coincidence of the retention times of the α-TA peaks in the chromatograms of the *test solution* and *reference solution*



Integration Results

	Name	RT	Start Time	End Time	Area	Height	% Area	Int Type
1	ThiocticAsid	8.949	8.517	9.767	7823165	593454	100.00	BV

Figure 2. *Chromatogram of the reference solution for the determination of thioctic acid*.

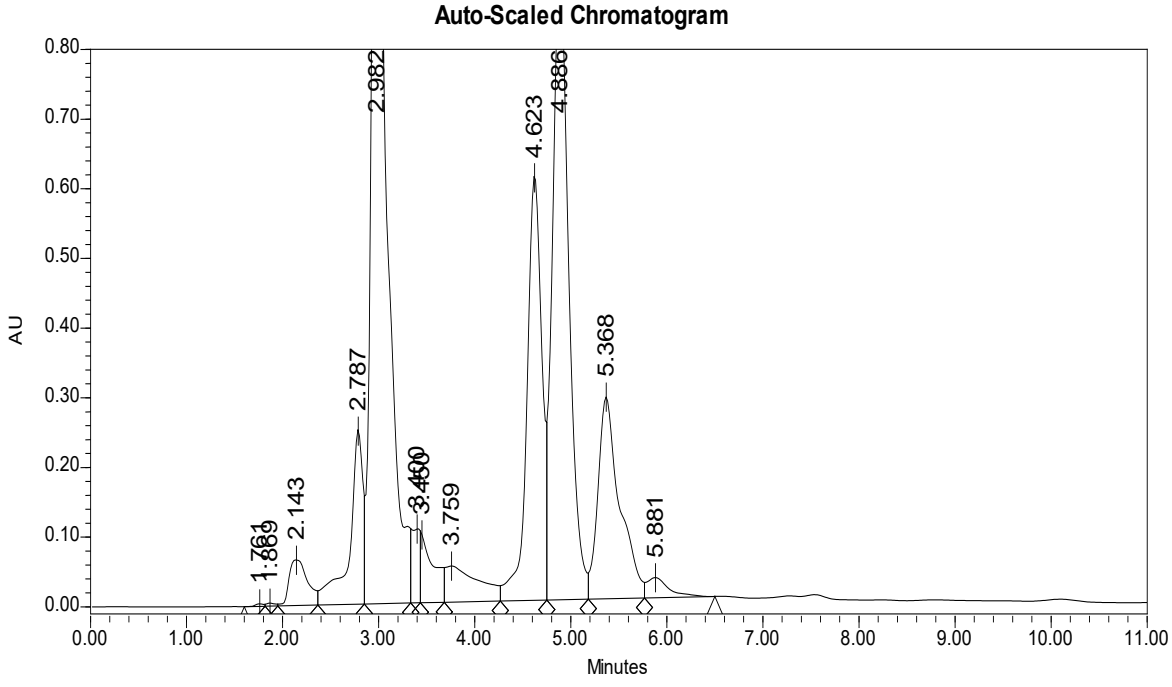


Figure 3*. Chromatogram of the "placebo" solution for the determination of thioctic acid.*

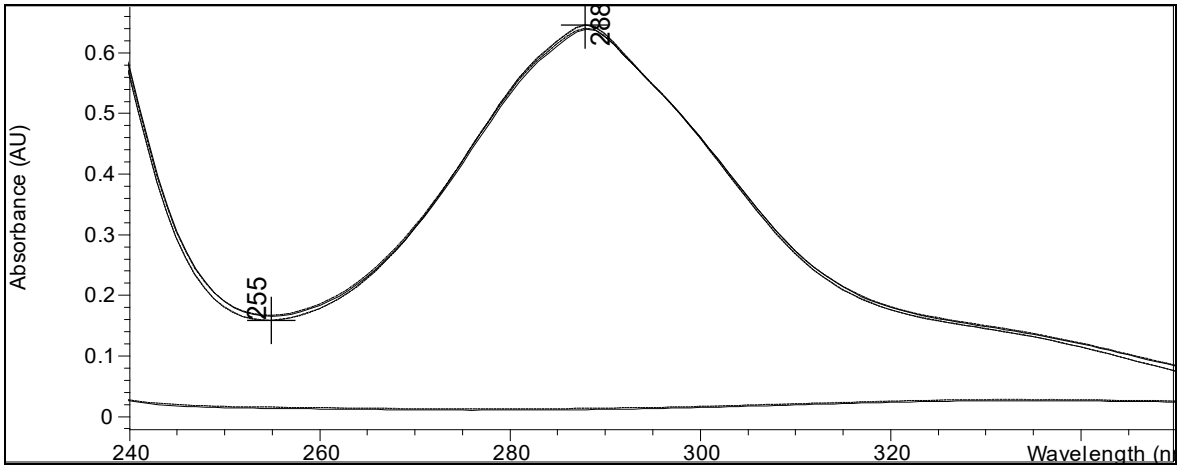


Figure 4. *UV spectra of the test solution, reference solution, and "placebo" solution for the quantitative determination of silymarin*

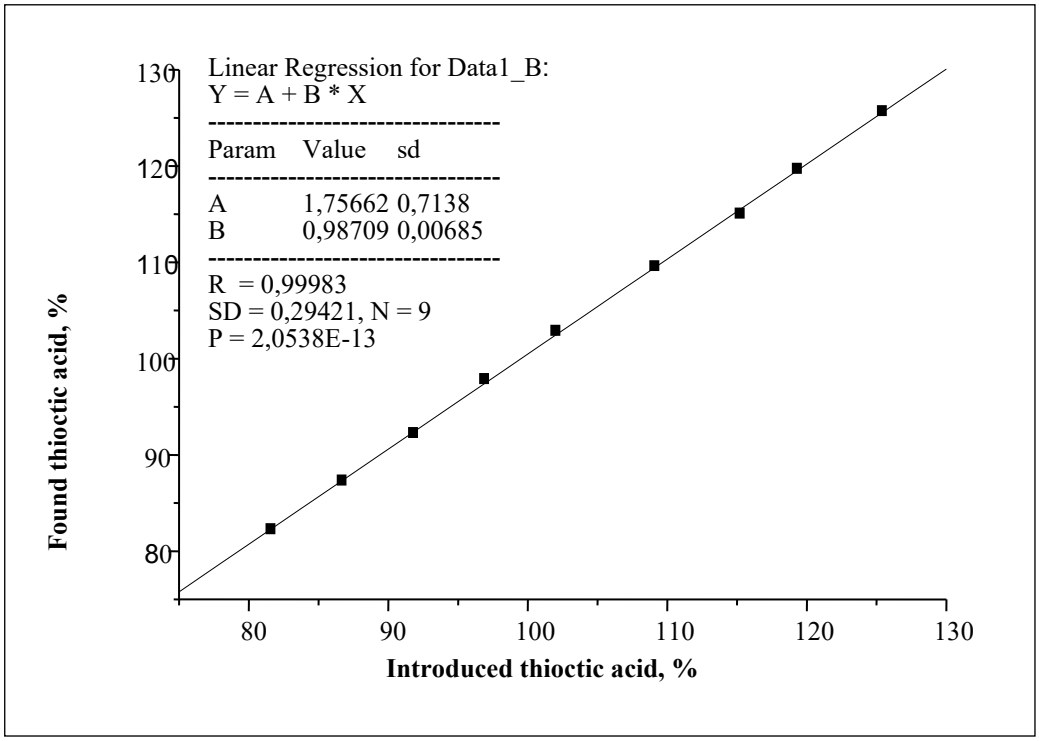


Figure 5. *Linear relationship between the peak area of thioctic acid and the concentration of thioctic acid in normalized coordinates*

confirms the identity of this compound. The resulting chromatograms are presented in Figures 1–3. The specificity of the test is confirmed by the following: the retention time of the α-TA peak in the *test solution* chromatogram (Figure 1) coincides with the retention time of the same peak in the *reference*

Table 1. Results of the analysis of model mixtures and their statistical processing for the quantitative determination of thioctic acid

№ Model solution	Added, % of reference solution concentration ($X_i = C_i/C_{st}$, %)	Average peak area (S_i) (S_{st} = 7,397,015)	Found, % of reference solution concentration ($Y_i = S_i/S_{st}$, %)	Found, % of added ($Z_i = Y_i/X_i$, %)
1	81.63	6081937	82.22	100.72
2	86.73	6455791	87.28	100.63
3	91.84	6820555	92.21	100.40
4	96.94	7235706	97.82	100.91
5	102.04	7605981	102.83	100.77
6	109.14	8102858	109.54	100.37
7	115.25	8506874	115.00	99.78
8	119.35	8850259	119.65	100.25
9	125.45	9293462	125.64	100.15
			Mean, Z_{cp}, %	100.44
			Relative standard deviation, RSD_z, % $RSD_z(\%) = \sqrt{\frac{\sum_i (Z_i - \overline{Z})^2}{n-1}} \bullet \frac{100}{\overline{Z}}$	0,35
			Relative confidence interval, $\Delta\% = t(95\%, n-1) \bullet RSD_z = 1{,}860 \bullet RSD_z$, %	0.66
			Critical value for repeatability, ΔA_s, % (maximum uncertainty)	1.6
			Systematic error $\delta = \lvert Z_{cp} - 100 \rvert$	0.44
			Criterion for insignificance of systematic error: 1) $\leq \delta = \frac{\Delta\%}{\sqrt{n}} = \frac{\Delta\%}{3}$ =0.66/3= 0.22	***Does not comply***
			2) If criterion (1) is not met → 0.51 → (1): $\delta \leq \delta_{theor}$, i.e., the systematic error does not exceed the theoretical value (0.51)	***Complies***
			Overall conclusion on method accuracy:	***Valid***

solution chromatogram within ±2% (Figure 2); the selected chromatographic conditions allow the α-TA peak to be separated from the peaks of excipients ("placebo"), as shown in Figure 3.

For the identification of silymarin, a UV spectrophotometric method was used, which also allows the quantitative determination of this compound in the formulation. The silymarin solution in methanol exhibits an absorption maximum at a wavelength of 288 ± 2 nm in the 240–350 nm range. The presence of this maximum in the tested solution indicates the identity of the drug. Figure 4 shows the absorption spectra of the *test solution*, *reference solution*, and the "placebo" solution in ethanol.

For the identification of selenomethionine, a colorimetric reaction with ninhydrin was used.

Assay of α-TA

The assay of α-thioctic acid (α-TA) was performed using a liquid chromatograph with a UV detector. For the analysis, a chromatographic column packed

Table 2. Metrological characteristics of the linear dependence for thioctic acid

Parameter	Value	Criterion (for acceptance limits 95.0 – 105.0 %), g = 9)	Conclusion
b	0.98709	-	-
S_b	0.00685	-	-
a	1.75662	1) ≤ 1.895 · S_a =1.35 2) if condition (1) is not met, then ≤ 2.6	Meets the requirement
S_a	0.7138	-	-
S_r	0.29421	-	
r	0.99983	≥ 0.9981	Meets the requirement

Table 3. Specificity assessment results: background absorbance and δnoise (%)

Optical Density		$\delta_{noise}(\%) = \delta_{placebo}(\%) = \frac{A_{placebo}}{A_{st}} \bullet 100\%$	Criterion. $\delta_{\text{noise theor}}$ (%)
Placebo Solution ($A_{placebo}$)	Reference Solution (A_{st})		
0.001154	0.643834	0.18	0.51
0.001189	0.644775		
0.001198	0.644317		
Mean $A_{placebo}$= 0.001181	Mean A_{st} = 0,644309		

with *octadecylsilyl silica gel for chromatography R (5 μm)* – Waters Spherisorb ODS2, sized 250 × 4.6 mm, was used. The *mobile phase consisted of a mixture of 8 volumes of acetonitrile R1*, 41 volumes of a 0.7 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*, and 51 volumes of *methanol R*. Detection was carried out at a wavelength of 215 nm.

The chromatograms of the *reference solution, test solution*, and "placebo" solution are presented in Figures 1–3, respectively.

The suitability of the chromatographic conditions for α-TA determination in the drug is ensured by the inclusion of a "*System suitability*" test, which covers all essential requirements for the analytical system: the column efficiency, calculated for the α-TA peaks, must be at least 5000 theoretical plates; the symmetry factor of α-TA peaks must be no more than 1.8; and the relative standard deviation (RSD), calculated for the areas of α-TA peaks, must comply with the requirements of the State Pharmacopoeia of Ukraine (SPhU, 2.2.46)[18].

Validation

The quantitative determination method of thioctic acid in combined tablets by liquid chromatography was validated in accordance with the State Pharmacopoeia of Ukraine (SPhU) requirements on "Validation of Analytical Methods and Testing" [12]. The validation was based on the criterion that the applicability range of the proposed method should be at least ±20% of the nominal content, i.e., from 80% to 120%, using acceptance criteria for content allowances of ±5.0% and a maximum overall analytical uncertainty $\Delta A_s \leq 1.6\%$.

Table 1 presents the results of the analysis of model mixtures and their statistical processing for the evaluation of precision, accuracy, and linearity. Data from this table indicate that the method for α-TA

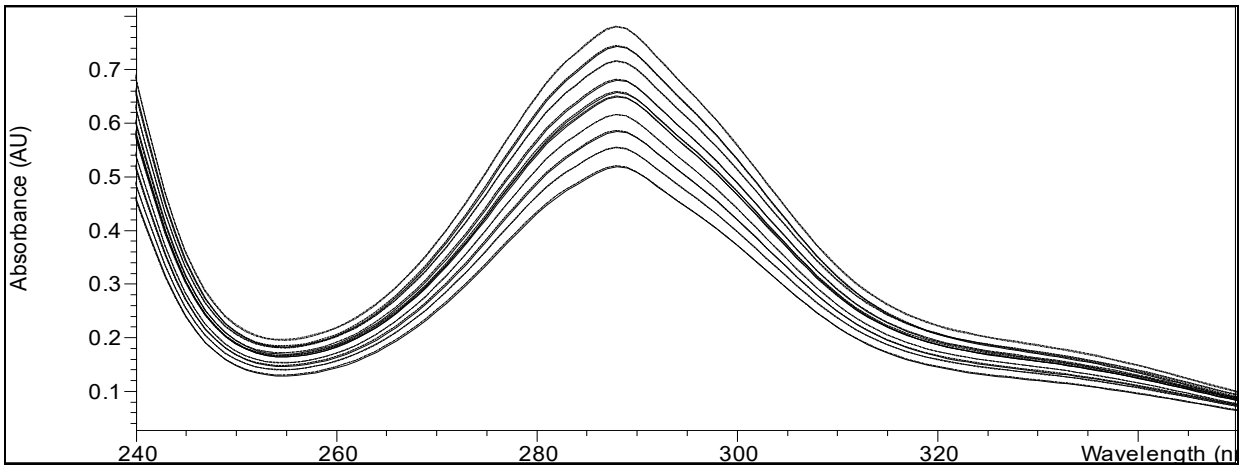


Figure 6. *UV spectra of standard solutions and the reference solution used to determine the linearity for the quantitative determination of silymarin*

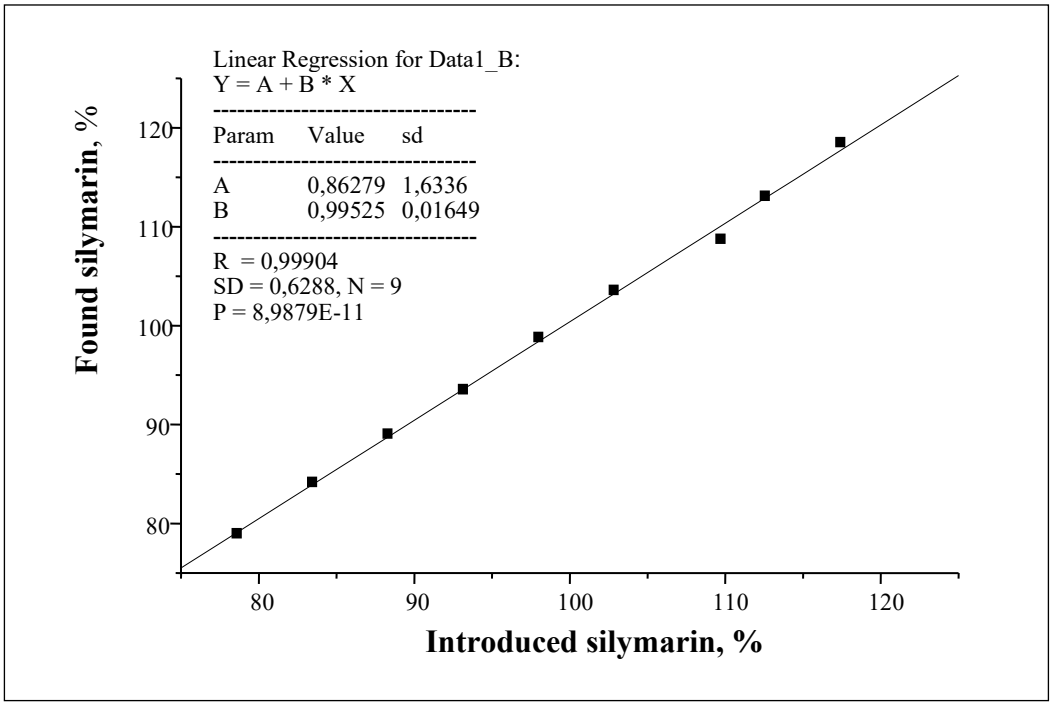


Figure 7. *Linear dependence of optical density on silymarin concentration in normalized coordinates*

demonstrates sufficient precision (repeatability) of the method. The calculated relative confidence interval (0.66%) is lower than the critical value for repeatability (1.6%).

The criterion of insignificance of the systematic error of the method is satisfied: the systematic error of 0.44% is practically negligible, which indicates that the analytical method demonstrates sufficient accuracy across the entire concentration range of 80–120% (Table 1).

Table 4. Results of the analysis of model mixtures and their statistical processing for the quantitative determination of silymarin

№ of model solution	Added, % of reference solution concentration (X_i= C_i/C_{st}, %)	Mean optical density (A_i) (A_{st} = 0.657403)	Found, % of reference solution concentration (Y_i= A_i/A_{st}, %)	Found, % of added concentration (Z_i = Y_i/X_i, %)
1	78.64	0.518820	78.92	100.36
2	83.49	0.553019	84.12	100.75
3	88.34	0.584957	88.98	100.72
4	93.19	0.614603	93.49	100.32
5	98.04	0.649251	98.76	100.73
6	102.89	0.680520	103.52	100.61
7	109.75	0.714472	108.68	99.02
8	112.60	0.743121	113.04	100.39
9	117.45	0.778652	118.44	100.84
			Mean Z_{mean}, %	100.41
			Relative standard deviation, RSD_z, % $RSD_z(\%) = \sqrt{\frac{\sum_i (Z_i - \overline{Z})^2}{n-1}} \bullet \frac{100}{\overline{Z}}$	0.55
			Relative confidence interval, $\Delta_t(\%) = t(95\%, n-1) \bullet RSD_z = 1{,}860 \bullet RSD_z$, %	1.03
			Critical value for result repeatability Δ_{As}, % (maximum uncertainty)	1.6
			Systematic error $\delta = \lvert Z_{cp} - 100 \rvert$	0.41
			3) Criterion for insignificance of systematic error	*Not met*
			$\leq \delta = \frac{\Delta\%}{\sqrt{n}} = \frac{\Delta\%}{3}$ =1.03/3= 0.34	***Met***
			4) To be applied if the requirement for criterion (1): $\delta \leq \delta_{theor}$ (0.51)	
			Overall conclusion on method accuracy	**Correct**

Thus, linearity, precision (repeatability), and accuracy of the determination of α-TA by the HPLC method in the working range of 80–120% have been confirmed.

The calculation of the parameters of the linear dependence $Y_i = b*X_i + a$ (based on the data in Table 1) was performed by the least squares method. The results are presented in Table 2, and Figure 5 shows the linear dependence of peak areas on α-TA concentration in normalized coordinates, which confirms compliance with the requirements for linear dependence parameters, i.e., the linearity of the method is confirmed over the entire concentration range of 80–120%.

Silymarin Assay.

The quantitative content of silymarin in tablets was determined using ultraviolet (UV) absorption spectrophotometry, with silybin employed as the reference standard. In methanol solution, both silymarin and silybin exhibit a maximum absorption at a wavelength of (228 ± 2) nm. The absorption spectra of the *test solution*, *reference solution*, and placebo

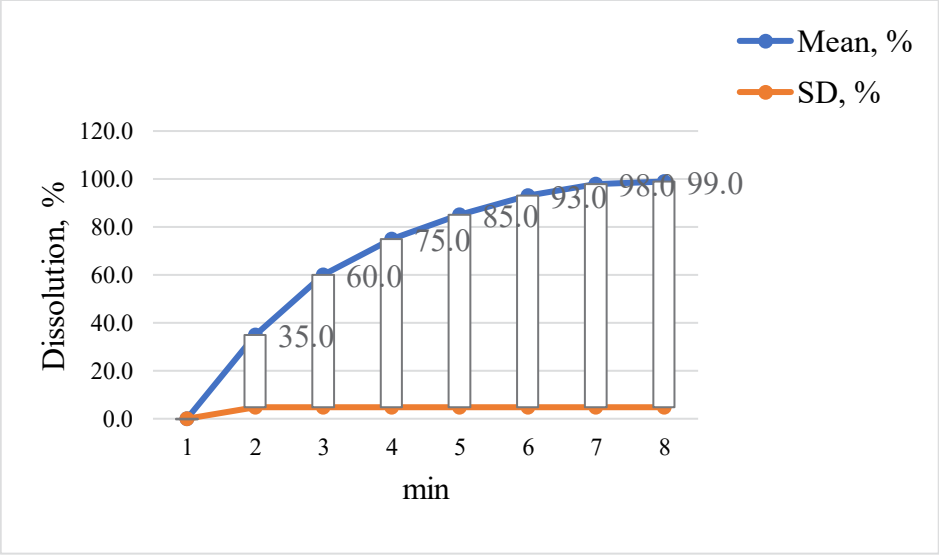


Figure 8. *Dissolution kinetic curve of silymarin from the developed combination tablets*

solution are presented in Fig. 4.

Validation

The method for the quantitative determination of silymarin in combined tablets by absorption spectrophotometry was also validated in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPU) section "Validation of Analytical Methods and Tests" [18], using acceptance criteria for content limits of ±5,0 % - Δ_{As} ≤. 1.6 %.

Specificity

To assess the specificity of the method, the background absorbance (A) and the relative systematic error δ_{noise} (%), (%) introduced by excipients were evaluated. The data are presented in Table 3.

The data in Table 3 show that the relationship δ_{noise} (%) ≤ $\delta_{noise\ theor}$ (%), is fulfilled, indicating that the background absorbance is not significant and the method possesses adequate specificity.

Accuracy, Precision, Linearity, and Applicability Range of the Method

The working concentration of silymarin is approximately 16 μg/mL. Linearity of the dependence of optical density on silymarin concentration was established in the range of ~12.8–19.2 μg/mL. UV spectra of the standard solutions and the reference solution used to determine linearity are presented in Fig. 6. Fig. 7 shows the linear dependence of optical density on silymarin concentration in normalized coordinates.

Table 4 presents the results of the analysis of model mixtures and their statistical processing for the assessment of precision, accuracy, and linearity. Data from these tables indicate that the analytical method for silymarin demonstrates sufficient precision (repeatability). The obtained value of the relative confidence interval (1.03%) is below the critical value for result repeatability (1.6%).

The results presented in Table 4 indicate that the an-

alytical method for silymarin exhibits sufficient repeatability and accuracy across the entire concentration range of 80–120%.

Selenium Assay.

According to USP-24, the quantitative content of selenium in selenomethionine substance is determined, which should range from 39.0% to 41.0%, using atomic absorption spectrometry. The determination of selenomethionine in the pharmaceutical preparation based on selenium is proposed to be carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Eur. Ph., 2.2.22).

Dissolution Testing of Silymarin. According to Eur. Ph., 2.9.3, for solid dosage forms containing multiple active substances, the dissolution specification may be established for only one active ingredient, provided that the other active ingredients have a higher dissolution rate. The formulation contains very slightly water-soluble α-lipoic acid, practically water-insoluble silymarin, and selenomethionine, which is soluble in hot water. Therefore, silymarin was selected for the dissolution test. For the development of the dissolution test, a 0.5% sodium dodecyl sulfate solution was used as the dissolution medium [19, 20]. The quantitative content of silymarin in the dissolution medium was determined by UV–visible spectrophotometry at a wavelength of 288 nm. Fig. 8 presents the dissolution kinetic curve of silymarin from the developed tablets.

The release of nicotinamide and calcium pantothenate follows a rapid diffusion mechanism due to their high water solubility. In contrast, the release of silymarin is governed by a combined mechanism of matrix erosion and diffusion, facilitated by the presence of superdisintegrants which ensure the optimal surface area for dissolution.

It was established that all tested samples of the combination tablets comply with Eur. Ph., 2.9.3 requirements for silymarin dissolution, namely that within 45 minutes, each of the six tablets releases at least 75% (Q) of the silymarin content relative to the nominal amount.

The silymarin release profile from the studied dosage form indicates rapid and complete dissolution of the active substance. Within just 10 minutes, approximately 60% of silymarin is released, which may be due to the presence of a portion of the substance in a highly dispersed or amorphous state, as well as the use of excipients with surfactant properties that facilitate wetting and particle dispersion. The subsequent increase in the dissolution degree reflects the gradual release of the residual fraction, which is limited by matrix erosion and diffusion processes.

Kinetic analysis suggests that the dissolution process can be described by a first-order model or the Higuchi model. The drug release profile from the developed tablets is characterized by a rapid initial phase for nicotinamide and calcium pantothenate, which follows a rapid diffusion mechanism due to their high water solubility. In contrast, for silymarin (BCS Class II/IV), the release mechanism is predominantly controlled by the erosive-diffusive process of the tablet matrix and the solubilizing effect of the selected excipients (cross-linked polymers). The synchronization of the components' release ensures the optimal synergistic hepatoprotective effect. The standard deviations (SD) of the release values did not exceed 5%, confirming the high reproducibility of the results.

4. Conclusion

The pharmaceutical development of the original combined hepatoprotective drug in tablet form has been analytically supported through a comprehensive study of the physicochemical properties of its active components. A sensitive and specific HPLC methodology was developed and validated for the simultaneous identification and quantification of silymarin, nicotinamide, and calcium pantothenate. The optimal composition of excipients, including fillers and disintegrants, was justified, providing the required technological characteristics of the tablets. In vitro dissolution studies confirmed the high efficiency of the drug release profile, and stability tests demonstrated the reliability of the developed formulation under various storage conditions. These results provide a solid analytical foundation for the further clinical introduction of the new hepatoprotective combination.

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