OPTIMIZATION OF THE METHOD FOR OBTAINING NANOCAPSULES, DEVELOPMENT OF THE METHODS OF DETERMINING THE DEGREE OF CINNARIZINE INCLUSION IN A PROLONGED DOSAGE FORM BASED ON POLY-D, L-LACTID-CO-GLICOLIDE, AND ITS VALIDATION

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Common pathologies of the cardiovascular system are cerebrovascular disorders, for which cinnarizine is prescribed. An innovative, prolonged nanocapsule dosage form based on poly-D-lactide-co-glycolide (PLGA) has been developed. The aim of the research was improvement of the technology and development of the methods for determining the level of cinnarizine inclusion in nanocapsules. The research problem consisted of a great number of drug encapsulation peculiarities, as well as various physicochemical properties of the substances that cannot be taken into account in the existing methods of determination.

Materials and methods. In the study, the following substances were used: cinnarizine, PLGA (50:50), polyvinyl alcohol (PVA). The remaining reagents and solvents fitted into the category of chemically pure. For development of the methods for quantitative determination of cinnarizine and its validation, a spectrophotometric method of analysis was used. Model mixtures used as objects of the study, had been prepared. Validation assessment of the methods was carried out upon such indicators as specificity, linearity, detection limit, repeatability, reproducibility.

Results. Methods for spectrophotometric determination of the degree of cinnarizine inclusion has been developed. It has been established that encapsulation reaches 63.74%. Validation testing methods has been carried out. The results of such tests as specificity, linearity, detection limit, repeatability, reproducibility correspond to the safe range of values regulated by Product specification file. Due to the impossibility of determining the degree of cinnarizine inclusion on the basis of standard methods of preparation, the technology of producing nanocapsules has been adjusted.

Conclusion. The technology has been optimized and new techniques have been developed. Taking into account the characteristics of production and the physicochemical properties of the components, they make a reliable analysis of the degree of cinnarizine inclusion in nanocapsules possible. The relative error of the developed methods of determination does not exceed ± 2.67%. Based on the results of the validation assessment, this methods is valid for all indicators.

Keywords: cinnarizine, poly-D-L-lactide-co-glycolide, microparticles, degree of incorporation, UV spectrometry


ОПТИМИЗАЦИЯ СПОСОБА ПОЛУЧЕНИЯ НАНОКАПСУЛ, РАЗРАБОТКА МЕТОДИКИ ОПРЕДЕЛЕНИЯ СТЕПЕНИ ВКЛЮЧЕНИЯ ЦИННАРИЗИНА В ПРОЛОНГИРОВАННУЮ ЛЕКАРСТВЕННУЮ ФОРМУНУ ОСНОВЕ ПОЛИ-D,L-ЛАКТИД-КО-ГЛИКОЛИДА И ЕЕ ВАЛИДАЦИЯ

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Распространенными патологиями сердечно-сосудистой системы являются цереброваскулярные расстройства, для лечения которых назначают препарат циннаризин. Разработана инновационная пролонгированная лекарственная форма в виде нанокапсул на основе поли-D,L-лактид-ко-гликолид (PLGA).

Цель исследования явилось совершенствование технологии и разработка методики определения уровня включения циннаризина в нанокапсулы. Исследовательская проблема заключалась в наличии множества особенностей инкапсулирования препарата, а также различных физико-химических свойств веществ, которые невозможно учесть в имеющихся методиках определения.

Материалы и методы. В ходе исследования были использованы субстанция циннаризина, PLGA (50:50), поливиниловый спирт (ПВС). Остальные реактивы и растворители соответствовали категории х.ч. Для разработки методики количественного определения циннаризина и ее валидации, использован спектрофотометрический метод анализа. Были приготовлены модельные смеси, которые использовались как объекты исследования. Валидационную оценку методики проводили по таким показателям, как специфичность, линейность, предел обнаружения, повторяемость, воспроизводимость.

Результаты. Разработана методика спектрофотометрического определения степени включения циннаризина. Установлено, что инкапсуляция составляет 63,74%. Осуществлено валидационное тестирование методики. Результаты таких испытаний, как специфичность, линейность, предел обнаружения, повторяемость, воспроизводимость соответствуют допустимому интервалу значений, регламентированных нормативной документацией. Скорректирована технология получения нанокапсул, в связи с невозможностью определения степени включения циннаризина при использовании стандартной методики получения.

Заключение. Оптимизирована технология и разработана методика, позволяющая провести достоверный анализ степени включения циннаризина, с учетом особенностей производства и физико-химических свойств компонентов. Относительная погрешность разработанной методики определения не превышает ±2,67%. Исходя из результатов валидационой оценки, по всем показателям методика валидна.

Ключевые слова: циннаризин, поли-D,L-лактид-ко-гликолид, микрочастицы, степень включения, УФ-спектрометрия

INTRODUCTION
In cerebrovascular disorders, progressive neuronal damage is observed, followed by their apoptosis due to metabolic disorders, nutritional deficiencies and oxygen. These are hemorrhagic and ischemic kinds of stroke, encephalopathy, migraine, dementia, cognitive impairment. One of the leading pathogenetic factors in the development of these disorders is a change in the properties of cerebral vessels under the influence of such factors as atherosclerosis, arterial hypertension, leading to impaired cerebral circulation [1–4]. The main group of drugs prescribed for this pathology, are calcium channel blockers, in particular cinnarizine, the effectiveness of which has been proven by numerous clinical studies [5]. Existing dosage forms of cinnarizine have several disadvantages, namely: frequency of administration and adverse effects [6, 7]. The solution to leveling these undesirable factors is developing a prolonged form of cinnarizine, allowing to reduce the frequency of administration, and thus avoid abrupt changes in the concentration of the drug in the body, and hereby to maintain the concentration of the drug in the body relatively constant and therapeutically effective. Modern pharmaceutical technologies make it possible to create prolonged dosage forms of nano- and microsized with controlled release of active substances based on polymeric carriers using the encapsulation method [8, 9]. One of the promising polymers is a copolymer of lactic and glycolic acids (50:50) – poly-D, L-lactide-co-glycolide (PLGA). Nanocapsules based on this biopolymer provide a high level of
sorption, prolonged and programmed release of drugs. The polymer is also biocompatible and biodegradable, it is metabolized in the body to endogenous compounds: lactic acid (decomposes to carbon dioxide and water) and glycolic acid (excreted unchanged). An innovative prolonged dosage form of cinnarizine in the form of micro- and nanocapsules has been developed. It is based on biodegradable polymer PLGA, supposedly capable of leveling the adverse effects of the drug.

**THE AIM** of the investigation was the adjustment of the standard technology, the development and validation of the methods for determining the degree of cinnarizine inclusion in the resulting microparticles, taking into account the features of encapsulation and the physicochemical properties of the compounds used in their production.

**MATERIALS AND METHODS**

In this study, the substance of cinnarizine, PLGA (50:50) and PVA have been used. The rest of the reagents and solvents fitted into the category of chemically pure.

1. **Methods for determining the degree of cinnarizine inclusion in nanocapsules**

   **Methods 1**

   The object of the study was an aqueous solution of the supernatant obtained after centrifugation [10–14]. The supernatant was placed in a 100 ml volumetric flask, adjusted to the mark with purified water (solution A). Considering the solubility of cinnarizine in ethyl alcohol and the literature data on the determination of the degree of incorporation of medicinal substances in PLGA-based microcapsules, the following methods was proposed.

   The degree of cinnarizine inclusion was established spectrophotometrically [15–21]. To do this, the content of free cinnarizine, and then the content of cinnarizine included in the microparticles were determined, A 0.4 ml aliquot was taken from solution A and placed in a 10 ml volumetric flask, made up to the mark with ethyl alcohol 95% [22–24].

   **Methods 2**

   A 20 ml aliquot was taken from solution A, placed in a separatory funnel, then 5 ml of chloroform was added and extracted. The spectrum of chloroform extraction was measured in the range of 220–360 nm on an SF-56 spectrophotometer in cuvettes with a layer thickness of 1 cm; chloroform was used as a reference solution.

   **Method 3**

   In this case, the object of the study was the solution of the supernatant in hydrochloric acid. The entire volume was transferred into a volumetric flask with a capacity of 50 ml, adjusted to the mark with a 0.1 M solution of hydrochloric acid. Previously, the spectrum of a standard sample of cinnarizine in a 0.1 M solution of hydrochloric acid was measured; an absorption maximum A = 0.576 at the wavelength of 253 nm was observed.

   The evaluation of the degree of cinnarizine inclusion in nanocapsules was performed by measuring the absorption spectrum in the range of 220–360 nm on an SF-56 spectrophotometer in cuvettes with a layer thickness of 1 cm; a solution of hydrochloric acid 0.1M was used as a reference solution.

2. **Optimization of the method of obtaining nanocapsules**

   It is known from the literature data, that cinnarizine is soluble in a 0.1 M solution of hydrochloric acid. Consequently, a study on the solubility of PLGA in a 0.1 M solution of hydrochloric acid was carried out. For this purpose, the PLGA sample was placed in 50 ml of a 0.1 M solution of hydrochloric acid. While stirring for three hours, the polymer did not dissolve, and therefore, it was proposed to change the standard technology of nanocapsules.

   **Standard nanocapsules technology**

   To obtain PLGA-based cinnarizine microparticles, the coprecipitation method was used. Precise weights of cinnarizine and PLGA were dissolved in a small volume of chloroform, and then added dropwise to the aqueous solution of PVA, using an Ultra-Turrax T-18 apparatus (IKA, Germany) for homogenization. The resulting solution containing microparticles, was centrifuged at the rotation speed of 10,000 rp/m for 20 minutes. The submicrocapsule fluid – supernatant – was decanted.

   **Adjusted nanocapsules technology**

   Precise weights of cinnarizine and PLGA were dissolved in a small volume of chloroform, and then added dropwise to a 0.1 M hydrochloric solution of PVA, using an Ultra-Turrax T-18 apparatus (IKA, FRG) for homogenization. The resulting solution containing microparticles, was centrifuged at the rotation speed of 10,000 rpm for 20 minutes. The submicrocapsule fluid – supernatant – was decanted.

3. **Methods for quantitative determination of cinnarizine in nanocapsules and its validation**

   **3.1. Quantitative determination**

   Based on the data obtained in determining the level
of cinnarizine inclusion in nanocapsules, for the quantita-
tive analysis, the conditions corresponding to Method
3 were selected.

The measurements were carried out six times at the
room temperature, the data were averaged.

The calculation of the content of cinnarizine in the
supernatant produced, is shown by formula 1:

\[ X = \frac{A_1 \times C_{cm} \times W_1 \times W_a}{A_{cm} \times a \times V_a} \]  

(1),

where

- \( A_1 \) – optical density of the supernatant;
- \( C_{cm} \) – CO concentration of cinnarizine, g/ml;
- \( W_1, W_a \) – volume of volumetric flasks, ml;
- \( A_{cm} \) – optical density CO cinnarizine;
- \( a \) – amount of cinnarizine, g;
- \( V_a \) – aliquot volume, ml.

### 3.2 Validation of the methods of cinnarizine
quantitative determination in nanocapsules

The validation estimation of the developed methods
for the quantitative determination of cinnarizine in mi-
croparticles has been carried out. The developed meth-
odology is evaluated according to the following indica-
tors: specificity, linearity, detection limit, repeatability,
reproducibility.

**Determination of specificity**

When testing a technique for specificity, the influ-
ence of a solvent and excipients on the optical density of
a solution of a model mixture is determined.

The solvent used is a 0.1 M hydrochloric acid solu-
tion. Two solutions are prepared: the first is a solution of
a model mixture of microparticles with cinnarizine, the
second is a solvent and excipients.

**Determination of linearity methods**

The determination of the linearity of the methods
of cinnarizine quantitative analysis is carried out by a
spectrophotometric method. To do this, 6 parallel deter-
minations in the weights of a model mixture of micro-
particles are conducted.

**Preparation of model mixtures**

Samples of cinnarizine microparticles equal to 0.01 g,
0.02 g, 0.03 g, 0.04 g, 0.05 g and 0.06 g, respectively, are
placed in 100 ml volumetric flasks, dissolved in a 0.1 M
hydrochloric solution of chlorohydric acid, brought to the
mark with the same solvent, then mixed. The optical densi-
ty of the obtained solutions is measure on spectrophotom-
eter SF-56 at the maximum absorption at the wavelength
of 253 nm in a cuvette with a layer thickness of 10 mm.

**Determination of the detection limit**

When determining the detection limit, a model mix-
ture is prepared: 0.03 g of cinnarizine microparticles are
placed in a 100 ml volumetric flask, dissolved in a 0.1 M
hydrochloric acid solution and made up to the mark with
the same solvent (solution A).

From solution A, model solutions were prepared
with a cinnarizine concentration of 1·10^{-3} g/ml, 1·10^{-4} g/
ml, 1·10^{-5} g/ml. The determination was started with the
solution with the highest concentration, setting the con-
centration at which there would be no cinnarizine ab-
sorption maximum at the wavelength of 253 nm.

**Determination of convergence**

The technique of convergence is characterized by
such indicators as repeatability and reproducibility.

To determine convergence, a spectrophotometric
method of analysis in the wavelength range of 220–360
nm was used.

Repeatability is examined on a model sample of
a mixture of cinnarizine microparticles in 10 replica-
tions.

Reproducibility of the methods is estimated on five
samples of model mixtures in two replications.

### RESULTS AND DISCUSSION

Using methods of determination No 1, it was found
out that when ethyl alcohol is added to the aqueous
solution of the supernatant, white opalescence and tur-
bidity are observed. The reason for these phenomena is
the interaction of PVA with ethyl alcohol, in connection
with which this method cannot be used for the spectro-
photometric determination of the degree of cinnarizine
inclusion.

Using methods No 2, the quantitative content of cin-
arizine (1% of the introduced cinnarizine sample) was
detected in the supernatant, which is confirmed by solu-
bility of cinnarizine in water [25].

On the basis on the obtained results it was proposed
to change the standard technology of microparticles by
using a hydrochloric solution of PVA instead of an aque-
ous solution, which would make it possible to transfer
non-included cinnarizine into the solution. Then it would
be possible to determine the degree of its inclusion
[26, 27].

The use of methods No 3 allowed us to determine
the quantitative content of cinnarizine in the hydrochlo-
ic solution of the supernatant. Accordingly, it became
possible to calculate the degree of encapsulation of the
drug in microparticles from the difference in concentra-
tions.

Having made a series of determinations, the follow-
ing data were obtained.

Fig. 1 shows the UV absorption spectrum of the
solution of the supernatant obtained by the adjusted
technology. This UV absorption spectrum coincides with
the results of the previous studies.
According to the results of the study, the average optical density at the wavelength of 253 nm was 0.447.

The content of cinnarizine in the supernatant was calculated by formula 1.

\[ X = \frac{0.447 \cdot 0.001 \cdot 50 \cdot 10}{0.576 \cdot 0.0107 \cdot 1} = 36.26\% \]

By differences, the degree of cinnarizine inclusion can be calculated:

\[ 100\% - x = 100 - 36.26\% = 63.74\% \] (Tab. 1)

### Table 1 – Data on the degree of encapsulation

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Optical density of solution</th>
<th>Amount of unbound cinnarizine, %</th>
<th>Amount of bound cinnarizine, %</th>
<th>Metrological data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.425</td>
<td>34.48</td>
<td>65.52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.482</td>
<td>39.10</td>
<td>60.90</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.447</td>
<td>36.26</td>
<td>63.74</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.450</td>
<td>36.51</td>
<td>63.49</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.430</td>
<td>34.88</td>
<td>65.12</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.448</td>
<td>36.34</td>
<td>63.66</td>
<td></td>
</tr>
<tr>
<td>( \bar{X} )</td>
<td>0.447</td>
<td>36.26</td>
<td>63.74</td>
<td></td>
</tr>
</tbody>
</table>

Validation assessment of the methods

Determination of specificity

The obtained solutions were analysed by a spectrophotometric method. Fig. 2 shows the UV spectrum of a model mixture of cinnarizine microparticles. In the range of 230–270 nm, an absorption maximum characteristic of the preparation is observed. Fig. 3 shows the absorption spectrum of the second solution, consisting of a solvent and excipients. It is clearly seen that the absorption in the range of 230–270 nm is practically absent.

Judging by the obtained definition data, one can see the specificity of the developed methodology. [28]
Determination of linearity methods

According to the test results, a calibration graph for the dependence of the optical density value on the mixture sample was constructed; the calibration curve equation and the correlation coefficient were calculated. The graph is presented in Fig. 4.

The linearity index is characterized by a correlation coefficient, its value was 0.998875, which meets the requirement of not less than 0.995.

The calibration curve is subject to linear dependence. The correlation coefficient meets the specified requirements ND [28]. Therefore, it can be argued that a linear dependence is observed in the concentration range of cinnarizine, in a sample of a model mixture of microparticles 0.000345–0.002%.
Determining detection limit
The data obtained during the study are reflected in Table 2. The developed method allows determining the detection limit, the concentration at which the characteristic absorption for cinnarizine will not be observed at the wavelength of 253 nm. The detection limit of cinnarizine in microparticles has been established. It is $1 \times 10^{-4}$ g/ml.

Table 2 – Data on detection limit

<table>
<thead>
<tr>
<th>Concentration of cinnarizine in solution, g/ml</th>
<th>Optical density of solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{-3}$</td>
<td>0.4980</td>
</tr>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>0.0508</td>
</tr>
<tr>
<td>$1 \times 10^{-5}$</td>
<td>0.0048</td>
</tr>
</tbody>
</table>

Determining convergence
Convergence is characterized by such parameters as repeatability and reproducibility of the methods.

Repeatability is estimated by the indicator – coefficient of variation, hereby its value at the request of ND should not exceed 2.0% [28]. The data on repeatability of the methodology are given in Table 3.

The established coefficient of variation was 1.4%, which is within the range of acceptable values.

The test results, in terms of reproducibility, are shown in Table 4.
Table 3 – Data on repeatability of the methods

<table>
<thead>
<tr>
<th>No. of measurement</th>
<th>Optical density</th>
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<tbody>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>0.505</td>
</tr>
<tr>
<td>3</td>
<td>0.504</td>
</tr>
<tr>
<td>4</td>
<td>0.497</td>
</tr>
<tr>
<td>5</td>
<td>0.496</td>
</tr>
<tr>
<td>6</td>
<td>0.507</td>
</tr>
<tr>
<td>7</td>
<td>0.503</td>
</tr>
<tr>
<td>8</td>
<td>0.500</td>
</tr>
<tr>
<td>9</td>
<td>0.494</td>
</tr>
<tr>
<td>10</td>
<td>0.496</td>
</tr>
</tbody>
</table>

Average value 0.500
Degree of variation, % 1.4

Table 4 – Data on the reproducibility of the methods

<table>
<thead>
<tr>
<th>Sample number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
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<td>0.500</td>
<td>0.503</td>
<td>0.498</td>
<td>0.496</td>
</tr>
<tr>
<td>Researcher No.2</td>
<td>0.504</td>
<td>0.503</td>
<td>0.505</td>
<td>0.499</td>
<td>0.502</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.506</td>
<td>0.507</td>
<td>0.500</td>
<td>0.503</td>
<td>0.501</td>
</tr>
<tr>
<td>Researcher No.2</td>
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<td>0.507</td>
<td>0.507</td>
<td>0.501</td>
<td>0.503</td>
</tr>
</tbody>
</table>

The acceptability indicator was the coefficient of variation; its value, according to the requirements of ND, should not exceed 3% [28]. For 10 parallel measurements in two series, it was 0.60%, which does not go beyond the acceptable indicator of the acceptance criterion.

CONCLUSION

In the course of the research, the standard micro-particle technology based on biodegradable polymer PLGA was adjusted by using a 0.1M hydrochloric acid solution as a solvent. A method for determining the degree of cinnarizine inclusion in microcapsules by the UV spectrophotometry method has been developed.

The content of cinnarizine in microcapsules was 63.7% of the introduced cinnarizine sample. The relative error does not exceed ±2.67%. The validation of the above-developed methods has been performed according to the following indicators: specificity, linearity, detection limit, repeatability, reproducibility. According to the test results, the data obtained can be used to judge the validity of the methods.

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CONFLICTS OF INTEREST
The authors and peer reviewers of this paper report no conflicts of interest.

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