Development and Validation of a Spectrophotometric Procedure for Determining Silicon in Common Horsetail (Equisetum arvense L.) Herb

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ABSTRACT

Background: An accurate, simple and selective UV-spectrophotometric method was developed for the estimation of silicon in medicinal plant raw material – horsetail herb (Equisetum arvense L.) of Russian origin. Methods: The determination of total silicon content in terms of silicon dioxide (SiO₂) by the direct ultraviolet-visible (UV-Vis) spectrophotometry method (at a wavelength of 915 ± 5 nm) in the horsetail herb is based on the formation of yellow-colored silicomolybdic acid, and its further reduction to molybdenum blue. Results: Recoveries were found to be in the range of 100.2 to 105.1% and %RSD was less than 2%. Conclusion: The developed method is accurate, specific, precise within an interval 2-12% and suitable for the analysis of horsetail herb commercial samples. Key words: Equisetum arvense, Horsetail herb, Medicinal plant raw material, Silicon.

INTRODUCTION

Silicon is one of the most common elements on Earth. Its oxide, silicon dioxide (SiO₂) is the main component of inorganic natural objects: ores, rocks, soils, and the earth’s crust.¹ Silicon plays an equally important role in wildlife – it is part of the tissues of plants, animals, and humans. Exogenous silicon has a very beneficial effect on plants, in particular, it contributes to the growth of shoots.² The lack of silicon leads to a significant decrease in endurance and reproduction of plants.³ Silicon is vital not only for autotrophs but also for humans. According to modern concepts, silicon provides protective functions of the body, contributes to detoxification. It is necessary for the implementation of normal metabolism,⁴ the functioning of bone tissue,⁵ while being present in the human body in several forms.⁶

1. water-soluble inorganic compounds (orthosilicic acid and its salts), easily penetrating through the cell walls and easily eliminated from the body;
2. silicones and silicone complexes;
3. insoluble polymers such as, for example, polysilicic acid, silicates, quartz.

Every day a person needs about 5-20 mg of silicon. The lack of this element leads to a loss of elasticity of the connective tissue fibers, deterioration of the blood supply to the organs, and the formation of atherosclerotic plaques. When there is a silicon deficiency in the human body, an obvious solution is substitution therapy. It includes the use of drugs with a high content of silicon in a form that is accessible to absorption. The use of modern herbal remedies is preferable for this type of treatment. Since herbal medicines have several significant advantages compared to synthetic ones: less toxicity, polyvalence, mildness and duration of action.

Silicon enters the plants from the soil in the water-soluble uncharged monomolecular form of silicic acid (H₄SiO₄ at pH 2 to 9) through the root system.¹⁰ However, only a small number of higher plant taxa can accumulate silicon in significant quantities, depositing in tissues in the form of insoluble opals (silicon dioxide – SiO₂), called phytoliths.¹¹ Although this circumstance significantly reduces the number of plant species that could be used as a source of silicon. However, some plants can accumulate silicon in a form that is accessible to humans, for example, horsetail, which is widely used in official Russian medicine.¹¹ It is also included in the European and British Pharmacopoeia.¹²,¹³ Horsetail – a spore-bearing herb of the horsetail family – Equisetaceae, with a long quitch rhizome.¹⁷ It is distributed throughout Northern Eurasia, except for deserts and semi-deserts, as well as the Far North. Fruitless summer shoots are used as medicinal plant raw materials, the content of silicic acid derivatives can reach 4% and even more.¹⁴,¹⁵ In the horsetail herb there are several soluble forms:

1. forms associated with high molecular weight organic compounds (proteins, polysaccharides, etc.), they can reach 50% of the total silicon content;
2. forms of silicon associated with hydrophilic organic compounds (about 40% of the total silicon content).

Namely, the proportion of potentially bioavailable forms of silicon can reach 90% of the total silicon content in this type of raw material. Thus, a horsetail herb can be considered as a natural source of silicon. The latter necessitates the standardization of this herb. A Multifaceted Journal in the field of Natural Products and Pharmacognosy

pharmaceutical substance of plant origin by the index – “Silicon content”. However, there are still no effective, validated, relatively simple, suitable for routine analysis methods for determining the total silicon content in horsetail raw materials in the leading world pharmacopoeias.

Currently, the leading methods for determining high elemental contents (at the level of units and tens of percent) in natural objects are atomic emission spectrometry with inductively coupled plasma (AES-ICP) and flame atomic absorption spectroscopy (FAAS). 20,21 As a sample preparation method, the most widespread is the decomposition of the analyzed samples under microwave heating. 22,23 Nevertheless, the determination of silicon by these methods is considered difficult due to the special chemical properties of this element. For example, it is necessary to use highly toxic hydrofluoric acid (HF) to convert an analyte to a dissolved form under conditions of closed microwave systems. HF reacts with a glass burner of an atomic-emission spectrometer, as well as with a nebulizer material of an atomic absorption spectrometer, and leads to expensive equipment damage. The use of special injection systems for sample solutions containing hydrofluoric acid can significantly degrade the analytical and metrological characteristics of the technique, significantly increasing the cost of analysis. To neutralize HF, it is necessary to add boric acid, 24,25 which is also undesirable for the subsequent determination of silicon by methods using inductively coupled plasma as a source of an analytical signal, due to an increase in the salt background. Besides, when using HF to decompose silicon-containing samples, there is a high risk of silicon loss due to the formation of volatile silicon tetrafluoride (SiF₄) at the sample preparation stage. The use of HF and boric acid in the determination of silicon in medicinal plant raw materials is an important task.

From this point of view a promising procedure is spectrophotometric quantitation, when the analyte is determined in the form of intensely colored silicon-molybdenum blue, 26-31 which provides high sensitivity, and preliminary fusion of the material being analyzed can significantly reduce the risk of loss of the silicon being detected due to the formation of volatile compounds.

The purpose of this study is to develop and validate a simple, effective procedure for the quantitative determination of total silicon content in terms of silicon dioxide (SiO₂) by the ultraviolet-visible (UV-Vis) spectrophotometry method in the horsetail herb – a potential source of natural silicon. The developed procedure can be recommended for the standardization of the horsetail herb by the “Silicon content” index.

**MATERIALS AND METHODS**

**Devices and materials**

UV-Vis spectrophotometer Varian Cary-100 Scan (Varian Inc., USA), a muffle furnace Nabatherm L. (LT) 5/11 (Nabatherm GmbH, Germany), platinum crucibles with a lid (National analytical corporation, India) were used.

**Standard samples, reagent**

Laboratory water (type 1), ammonium molybdate qualification “HCH” (Chimmed, Russia), tartaric acid high grade purity (Chimmed), metol high grade purity (Chimmed), sodium sulfate anhydrous high grade purity (Chimmed), sodium tetraborate high grade purity (Chimmed), sodium carbonate anhydrous high grade purity (Chimmed), potassium nitrate high grade purity (Chimmed), hydrochloric acid concentrated high grade purity (Chimmed), Russian State Standard Sample (RSSS) 728-75 composition of nepheline ore SNS-1 (Chimmed).

**5% ammonium molybdate solution**

50 g of ammonium molybdate is dissolved in 500-600 ml of purified boiling water. After cooling, the solution is filtered in a 1000 ml plastic volumetric flask, the solution volume is adjusted to the mark with the same solvent.

**10% tartaric acid solution**

100 g of tartaric acid is placed in a 1000 ml volumetric flask, 700 ml of water is added, and stirred until the mixture is completely dissolved, solution volume is adjusted to the mark with the same solvent.

**Recovery solution**

20 g of colorless metol are dissolved in 800 ml of water, 13 g of anhydrous sodium sulfate are added, the resulting solution is filtered into a 1000 ml volumetric flask, and the solution volume is adjusted to the mark with water. The solution is stored in a dark flask no more than 8-10 days.

**Mixture for melting**

A) sodium tetraborate, dehydrated: To dehydrate sodium tetraborate, its portions of 8-10 g placed in porcelain cups are heated in a muffle furnace. The temperature is raised within 2 hours to 300-400°C and sodium tetraborate is kept at this temperature for 4 hours.

B) sodium carbonate anhydrous: To prepare the mixture for fusion, 2 parts by weight of anhydrous sodium carbonate and 1 part of dehydrated sodium tetraborate are carefully ground in porcelain mortar. The grinded mixture is thoroughly mixed in a glass jar with a ground-in lid until a homogeneous mixture is obtained.

**A solution of hydrochloric acid 1:3**

One volume of concentrated hydrochloric acid is diluted with three volumes of purified water.

**Standard stock solution**

For the preparation of the initial solution, use RSSS 728-75 of the nepheline ore composition CHC-1 (content of silicon dioxide (SiO₂) 40.2%) or another geochemical standard with a content of silicon dioxide (SiO₂) about 40%.

The exact amount of a standard sample weighing 100 mg is placed in a platinum crucible, thoroughly mixed with 3 g of the mixture for melting and 5-10 mg of potassium nitrate. Sample in a crucible is covered with a platinum lid and melted for 15-20 minutes in a muffle at a temperature of 900°C to obtain a liquid melt. Having taken out the crucible from the muffle, the cooling melt on the crucible walls should be distributed by rapid rotation. After cooling, the crucible and the lid are placed in a plastic cup with a volume of at least 300 ml and poured with such amount of boiling water that the crucible is covered with it, but not less than 100 ml. Stir with a plastic stick 3-4 times and leave overnight. After completing the disintegration of the melt, 100 ml of 1:3 hydrochloric acid solution is immediately added to the glass (not more than 2 glasses at the same time). Stirring and mixing are continued until the hydrates of the mixed oxides are completely dissolved. Then the solution is transferred to a 500 ml volumetric flask; glass, crucible, and lid are washed with purified water. The volume of the solution is adjusted to the mark with water and mixed.

**Reference solution**

Prepare similarly to the standard stock solution (see section “Standard samples, reagents”) without adding a standard or test sample.

**Sample information**

A sample of the horsetail herb was purchased in the pharmacy network of the city of Moscow to develop and validate a method for quantifying total silicon content in terms of silicon dioxide (SiO₂). This sample corresponded to the requirements of the State Pharmacopoeia of Russian Federation XIV edition.
Sample preparation
It is carried out similarly to the standard stock solution (see “Standard samples, reagents”), except that the mass of a sample of horsetail herb taken for analysis is 200 mg (exact weight).

Calibration curve
To obtain a calibration curve, solutions were used to study linearity (Table 1).

Procedure
5 ml of the test and reference solutions, 5 ml of 5% ammonium molybdate solution were taken in a 100 ml volumetric flask, mixed, left for 8-10 min for the development of yellow color. 5 ml of 10% tartaric acid solution were added, mixed. After 1-2 min 20 ml of recovery solution were added, mixed. The volume of the solution is adjusted to the mark with water, stirred, left for 2 hours.

The optical density of the standard and test solutions is measured at a wavelength of 815 ± 5 nm in a cuvette with a layer thickness of 10 mm relative to the reference solution.

The staining of the reference solution is carried out as follows: in a 100 ml volumetric flask, 10 ml of the reference solution, 5 ml of the 5% ammonium molybdate solution are taken, stirred, left for 8-10 min to develop a yellow color. 5 ml of the 10% tartaric acid solution is added, stirred. After 1-2 minutes, 20 ml of the recovery solution is added, mixed. The volume of the solution is adjusted to the mark with water, stirred, left for 2 hours.

Standard solutions are prepared according to Table 1 should stay at room temperature for 2 hours.

The total silicon content in terms of silicon dioxide (SiO₂) in percent (X, %) is calculated by the formula:

\[ X = \frac{A_{\text{std}} \times (SiO_2)_{100}}{A \times (100 - W)} \]

where

- \( \omega \) (SiO₂) – content of silicon dioxide (SiO₂), determined according to the calibration curve, %;
- \( a_0 \) – RSSS 728-75 of the nepheline ore composition CHC-1 (content of silicon dioxide SiO₂ – 40.2%), mg;
- \( a \) – weight of horsetail herb, mg;
- \( W \) – humidity of horsetail herb%.

Validation of an analytical procedure was carried out with the requirements of monograph 1.1.0012.15 “Validation of analytical methods” of State pharmacopoeia of Russian Federation that is harmonized with ICH Harmonised Tripartite Guideline “Validation of Analytical Procedures”.

Table 1: Preparation of silicon solutions for linearity studies.

<table>
<thead>
<tr>
<th>No</th>
<th>Volume of initial solution, ml</th>
<th>Volume of reference solution, ml</th>
<th>Volumes of reagents, ml</th>
<th>Content of silicon dioxide (SiO₂), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>9.50</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0.67</td>
<td>9.33</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>9.00</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1.50</td>
<td>8.50</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>2.00</td>
<td>8.00</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>3.00</td>
<td>7.00</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

*Volumes of initial and reference solutions are adjusted depending on the content of silicon dioxide (SiO₂) in RSSS

RESULTS AND DISCUSSION
Justification of methodology
The analytical procedure basis is presented in Gelman EM et al. and also Coradin T et al. researches. This procedure is distinguished by high sensitivity – the limit of quantification of total silicon content in terms of silicon dioxide (SiO₂) is 0.2%. The determination of silicic acid is based on the formation of yellow-colored silicomolybdic acid, and its further reduction to molybdenum blue. The blue complex differs from the yellow one by a smaller dependence of optical density on temperature. Unlike works where tin (II) chloride is used as a reducing agent, this procedure uses a metolsulfite reagent. It allows the reduction process to be carried out under mild conditions without contaminating the analyzed solutions with large quantities of foreign elements. The blue color of the recovered compound develops within 1-2 hours and is stable for at least 2 days. Calcium, magnesium, aluminum, iron, manganese, alkali and alkaline earth metals, nickel, cobalt, copper, zinc, lead, tin, bismuth, zirconium, rare earth metals, thorium, molybdenum, tungsten, vanadium, chromium do not interfere with the determination. Phosphorus, arsenic, and germanium interfere with the determination, also forming colored complex compounds with ammonium molybdate. Titanium forms a poorly soluble compound with ammonium molybdate. The addition of tartaric acid allows to effectively mask the interfering effects of elements without prior separation of the matrix. Moreover, tartaric acid allows destroying the poorly soluble compound of ammonium molybdate and titanium due to the formation of easily soluble complex compounds with it. When the content of silicon dioxide (SiO₂) is up to 40% in the analyzed samples, it is possible to determine the analyte at a wavelength of 815 ± 5 nm using direct spectrophotometry; it greatly simplifies the analysis procedure.

Since there are no pharmacognostic standards for silica-based medicinal plant raw materials, we chose the geochemical standard RSSS 728-75 of the nepheline ore composition CHC-1 with a silica content (SiO₂) of 40.2% as the standard. It should be noted that in the absence of this SiO₂ standard sample, any standard of SiO₂ with the appropriate conversion of silica content can be used.

Specificity
To assess the specificity the UV-Vis spectra of the reference solution (Figure 1), standard solutions used to build linearity (Figure 2), and the test solution (Figure 3) at \( \lambda = 818 \) nm were obtained.

The specificity of the method for determining total silicon content in terms of silicon dioxide (SiO₂) is presented in Table 2.

The UV-Vis absorption spectrum of the test solution prepared for quantification, in the range from 200 to 850 nm, corresponds to the
UV-Vis absorption spectrum of the standard solution. The absorption maxima are at 818 nm and coincide with an accuracy of more than 99%. The UV-Vis spectra of the reference solution indicate the absence of the influence of the matrix components on the results of the quantitative determination of total silicon content in terms of silicon dioxide ($\text{SiO}_2$).

**Limit of detection (LOD) and limit of quantification (LOQ)**

LOD is the lowest quantity of analyte in an analyzed sample, that can be detected but not obligatorily quantified as an exact value; LOQ is the lowest quantity of analyte in an analyzed sample, that can be determined quantitively with suitable accuracy and precision. Values of LOD (3.3\(\times\)σ/S) and LOQ (10\(\times\)σ/S) were 0.2% and 1.0% respectively (σ – standard deviation, S – slope of the calibration curve).

**Linearity**

To determine the linearity of the method for quantification of total silicon content (in terms of silicon dioxide ($\text{SiO}_2$)), a series of solutions were prepared by diluting the initial standard solution. The preparation of solutions for the study of linearity is shown in Table 1. Staining was performed according to the method described above.

An estimate of the linearity of the determination of total silicon content in terms of silicon dioxide ($\text{SiO}_2$) is given in Table 3.

According to the obtained data, a curve was obtained reflecting the dependence of optical density on the content of silicon dioxide ($\text{SiO}_2$) (Figure 4). The resulting curve is processed by the method of least squares.

The value of the correlation coefficients for the linear dependence is more than 0.995, which confirms the linearity of the method for the quantitative determination of total silicon content in terms of silicon dioxide ($\text{SiO}_2$).

The revealed linear dependence of the analytical signal on the content of silicon dioxide ($\text{SiO}_2$) can be used as a calibration curve in determining the analyte.

**Trueness (accuracy)**

The trueness of the method for the quantitative determination of total silicon content in terms of silicon dioxide ($\text{SiO}_2$) was proved by the method of additives (by adding the exact volume of the initial solution). Three parallel determinations were carried out for each additive, adding the initial solution to the analyzed solution, so that the final concentration of the analyte was in the linear concentration range (Figure 4). The trueness index was estimated by calculating the recovery, that is, as the ratio between the total content of the added silica ($\text{SiO}_2$) and the initially available silica ($\text{SiO}_2$) in the test solution, expressed as a percentage. The results of determining the trueness of the methodology are presented in Table 4.

The data presented show that the trueness of the quantitative determination of total silicon content in terms of silicon dioxide ($\text{SiO}_2$) for the average value of each of the three definitions is in the range from 95.0 to 105.0%, as required by acceptability criteria. Statistical characteristics are presented in Table 5.

The data obtained indicate that the coefficient of variation of the quantitative determination of the analyte (n = 9) does not exceed 4.0% and it is 1.98%.

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**Table 2: The specificity of the method for determining total silicon content in terms of silicon dioxide ($\text{SiO}_2$).**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Standard solution</th>
<th>Test solution</th>
<th>Coincidence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>818.0</td>
<td>818.0</td>
<td>100.0</td>
</tr>
<tr>
<td>2</td>
<td>818.0</td>
<td>820.0</td>
<td>100.2</td>
</tr>
<tr>
<td>3</td>
<td>818.0</td>
<td>818.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Figure 1:** Typical UV-Vis spectrum of comparison solution.

**Figure 2:** Typical UV-Vis spectrum of a standard solution with total silicon content in terms of silicon dioxide ($\text{SiO}_2$) – 4%.

**Figure 3:** Typical UV-Vis spectrum of the test solution.
**Table 3: Estimation of the linearity of the determination of total silicon content in terms of silicon dioxide (SiO\(_2\)).**

<table>
<thead>
<tr>
<th>No</th>
<th>A(_1)</th>
<th>A(_2)</th>
<th>A(_3)</th>
<th>A(_{\text{average}})</th>
<th>SiO(_2) content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1477</td>
<td>0.1475</td>
<td>0.1475</td>
<td>0.1475</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0.1968</td>
<td>0.1968</td>
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<td>0.1968</td>
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</tr>
<tr>
<td>3</td>
<td>0.2953</td>
<td>0.2952</td>
<td>0.2952</td>
<td>0.2952</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>0.4424</td>
<td>0.4428</td>
<td>0.4424</td>
<td>0.4425</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>0.5830</td>
<td>0.5830</td>
<td>0.5830</td>
<td>0.5830</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>0.8849</td>
<td>0.8852</td>
<td>0.8849</td>
<td>0.8850</td>
<td>12</td>
</tr>
</tbody>
</table>

*Optical density

**Table 4: The trueness of total silicon content in terms of silicon dioxide (SiO\(_2\)) determination in horsetail herb (a = 0.357 relative units)*.

<table>
<thead>
<tr>
<th>Additive content, %</th>
<th>Optical density of the additive, relative units</th>
<th>Expected optical density, relative units</th>
<th>Found, relative units</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.074</td>
<td>0.431</td>
<td>0.438</td>
<td>101.6</td>
</tr>
<tr>
<td>1.0</td>
<td>0.075</td>
<td>0.432</td>
<td>0.437</td>
<td>101.2</td>
</tr>
<tr>
<td>1.0</td>
<td>0.074</td>
<td>0.431</td>
<td>0.438</td>
<td>101.6</td>
</tr>
<tr>
<td>2.0</td>
<td>0.148</td>
<td>0.505</td>
<td>0.506</td>
<td>100.2</td>
</tr>
<tr>
<td>2.0</td>
<td>0.147</td>
<td>0.504</td>
<td>0.506</td>
<td>100.4</td>
</tr>
<tr>
<td>2.0</td>
<td>0.148</td>
<td>0.505</td>
<td>0.507</td>
<td>100.4</td>
</tr>
<tr>
<td>3.0</td>
<td>0.198</td>
<td>0.555</td>
<td>0.581</td>
<td>104.7</td>
</tr>
<tr>
<td>3.0</td>
<td>0.195</td>
<td>0.552</td>
<td>0.580</td>
<td>105.1</td>
</tr>
</tbody>
</table>

*In parentheses are data for silicon contained in the test solution

**Table 5: Statistical characteristics of recovery.**

<table>
<thead>
<tr>
<th>Statistical characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest%</td>
<td>100.2</td>
</tr>
<tr>
<td>The highest value, %</td>
<td>105.1</td>
</tr>
<tr>
<td>Average value, %</td>
<td>102.7</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.03</td>
</tr>
<tr>
<td>Standard deviation of the average result</td>
<td>0.68</td>
</tr>
<tr>
<td>Coefficient of variation (CV),%</td>
<td>1.98</td>
</tr>
<tr>
<td>Confidence interval (P = 0.95)</td>
<td>1.56</td>
</tr>
</tbody>
</table>

**Repeatability**

To determine the repeatability, the coefficient of variation was calculated from the results of the quantification of total silicon content in terms of silicon dioxide (SiO\(_2\)) (n = 6) in the test solution. The results are shown in Table 6.

Analysis of the data presented in Table 6 shows that the coefficient of variation of the results of the quantitative determination of total silicon content in terms of silicon dioxide (SiO\(_2\)) in the horsetail herb (n = 6) is 1.50%.

**Range**

The method of quantitative determination of total silicon content in terms of silicon dioxide (SiO\(_2\)) is applicable in the range of contents from 2 to 12% of the analyte in the test solution. Trueness, linearity, repeatability, interlaboratory precision are determined for interval values and values within an interval: linearity (2-12%), accuracy (2-12%), repeatability and intralaboratory precision (about 5.66%).

In the State pharmacopoeia of the Russian Federation (14th edition), the state pharmacopoeia of the Republic of Belarus, as well as in the British...
An effective technique has been developed for the quantitative determination of total silicon content in terms of silicon dioxide (SiO\textsubscript{2}). The technique can also be recommended for inclusion in leading world pharmacopoeias.

ACKNOWLEDGEMENT

The publication has been prepared with the support of the «Russian Academic Excellence Project 5-100» («RUDN University Program 5-100», «Sechenov First Moscow State Medical University Program 5-100»).

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

REFERENCES

GRAPHICAL ABSTRACT

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