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The method of obtaining a kit for the Technetium-99m Generator based on d,l-hexamethylpropyleneamine oxime (HM-PAO) with divalent tin, for the preparation of a diagnostic agent

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ABSTRACT: A technology has been developed for producing a kit of d,l-hexamethylpropyleneamine oxime (HMPAO) with divalent tin (HMPAO-Sn(II)) for the Technetium-99m Generator, which guarantees consistently high quality characteristics with sterility and pyrogen-free final product in the form of a lyophilisate. Studed the influence of ligand concentration, Sn^{2+} ion concentration, and pH on the efficiency of HMPAO^{99m}Tc complex formation in solutions, a series of HMPAO-Sn(II) complexes was synthesized with varying ligand concentrations ranging from 0.35 to 0.6 mg/ml, Sn^{2+} ion concentrations from 0.35 to 0.7 μg/ml, and pH values between 9.0 and 10.0. The obtained HMPAO-Sn(II) complexes in the form of a lyophilized powder were dissolved by adding 3.0 ml of sodium pertechnetate ($Na^{99m}TcO_4$) solution, obtained from a technetium-99m generator. Immediately after dissolving the lyophilized powder, the amount of formed HMPAO^{99m}Tc complex was determined, and the unbound $99mTcO_4^-$ and hydrolyzed $99mTcO_2$ were identified using paper and thin-layer chromatography methods. The formation efficiency of the stable HMPAO^{99m}Tc complex was established to be more than 90.0% in kits containing: Sn^{2+} 0.5 μg /ml and HMPAO 0,4-0,45 mg/ml and solution pH 9.5, after 5 minutes adding to the kit radioactive $^{99m}TcO_4^-$. The stability of the kit HMPAO-Sn(II) stored for 12 months was determined by obtaining the radiopharmaceutical HMPAO^{99m}Tc. Also, a draft regulatory and technical documentation for quality control has been developed.

KEYWORDS: radiopharmaceutical; substance; radionuclide; technetium-99m.

I. INTRODUCTION

Technetium-99m (^{99m}Tc) is one of the most widely used radionuclides in nuclear medicine, owing to its outstanding physicochemical properties, such as a short half-life (6 hours) and gamma radiation energy of 140 keV, which allows for high sensitivity and resolution in imaging with minimal radiation exposure to the patient. These properties make ^{99m}Tc ideal for a wide range of diagnostic procedures, minimizing the radiation burden [1-2].

The method of producing radiopharmaceuticals (RPh) based on ^{99m}Tc involves extracting technetium from ^{99}Mo/^{99m}Tc generators and then combining it with functional ligands, which provide specificity and targeting for the radiopharmaceutical. In particular, ^{99m}Tc-containing radiopharmaceuticals using the ligand d,l-hexamethylpropyleneamine oxime (HMPAO), such as HMPAO^{99m}Tc, play a key role in diagnosing central nervous system diseases. They are used for visualizing conditions such as transient ischemia of the brain, strokes, epilepsy, migraines, dementia, tumors, and inflammatory processes of various localizations [3-6].

HMPAO^{99m}Tc allows for high-precision imaging with detailed visualization of both vascular and tissue changes in the brain, as well as providing accurate assessment of the functional state of specific brain regions. This is particularly important for evaluating ischemic zones, tumor processes, and neurodegenerative changes.

The production of similar radiopharmaceuticals is carried out by leading global companies such as Medi-Radiopharma LTD (Hungary), GE HealthCare, (USA), China Isotope & Radiation Corporation (China) and Diamed

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(Russia), which control a significant portion of the medical radionuclide market and ensure their widespread clinical use. These companies are actively engaged in developing new forms of radiopharmaceuticals and improving their manufacturing technologies, which contributes to enhancing diagnostic quality approaches in nuclear medicine.

The goal of this work is to develop a technological process for obtaining a reagent kit in the form of a lyophilisate for a domestic radiopharmaceutical HMPAO^{99m}Tc. Within this approach, the optimization of the preparation process is aimed at reducing losses of both raw materials and the complexing agent. It is expected that the proposed technology will simplify and accelerate the synthesis process, improve the yield of the product, and enhance its stability, which, in turn, will ensure economic efficiency and high-quality final products.

II. METHODS, MATERIALS, REAGENTS AND EQUIPMENT.

The following chemical reagents were used in the preparation of the kit HMPAO -Sn(II):
- ligand d,l-hexamethylpropyleneamine oxime (HMPAO),, purity not less than 98%, Diamed (Russia);
- tin dichloride, purity not less than 99%, Sigma Aldrich;

Radiometric measurements of the samples were carried out on a gamma spectrometer with a semiconductor Ge(Li), detector and Aspect software using a spectrometric device SU-03P No. 0037-06. The preparation of experimental batches of the kit HMPAO -Sn(II) was carried out in a reaction unit. Solutions of the kit dispensed in vials with a capacity of 10 cm^3, were lyophilized in the sublimation devices Epsilon2-16D. Qualitative and quantitative characteristics of the main substance d,l-hexamethylpropyleneamine oxime (HMPAO) and excipients were determined by the spectrophotometric method on the Genesys 10S UV-Vis spectrophotometer, (Thermo scientific, USA).

III. METHOD OF OBTAINING A KIT BASED ON d,l -HEXAMETHYLPROPYLENEAMINE OXIME (HMPAO) AND THE REDUCING AGENT Sn^{2+}.

Figure 1 shows the device designed for the synthesis of a complex compound based on the ligand HMPAO with divalent tin, used for obtaining a lyophilized product necessary for the Technetium-99m Generator. The device consists of several key components, each of which plays an important role in the synthesis and lyophilization process. Description of the Device: Gas Purification Vessel (1), this container holds the purification mixture for preparing argon. The purified argon supplied to the system is necessary to create an inert atmosphere during the synthesis process, preventing unwanted reactions with oxygen; Beaker for synthesis of the substance solution (2), this vessel is where the chemical reaction takes place. HMPAO dissolves here, and components such as sodium chloride and mannitol are added, which facilitate the necessary processes for the synthesis; Magnetic stirrer (3), this device is used to evenly mix the solution, ensuring the necessary homogeneity of the mixture and activating the chemical reactions required for the formation of the complex compound; Argon supply tube (4), this pipe supplies purified inert gas, argon, to the reaction chamber. It is essential for preventing contact with oxygen during the synthesis process; Membrane filter attachment (5), this filter, with a pore size of 0.22 µm, is installed in a conical flask with a drain. It is used to purify the obtained solution from impurities before directing the solution to the vacuum system for collection; Flask for collecting the finished product (6), this flask collects the complex compound solution. It is connected to a vacuum pump that creates the necessary pressure for filtration and further removal of solvents.

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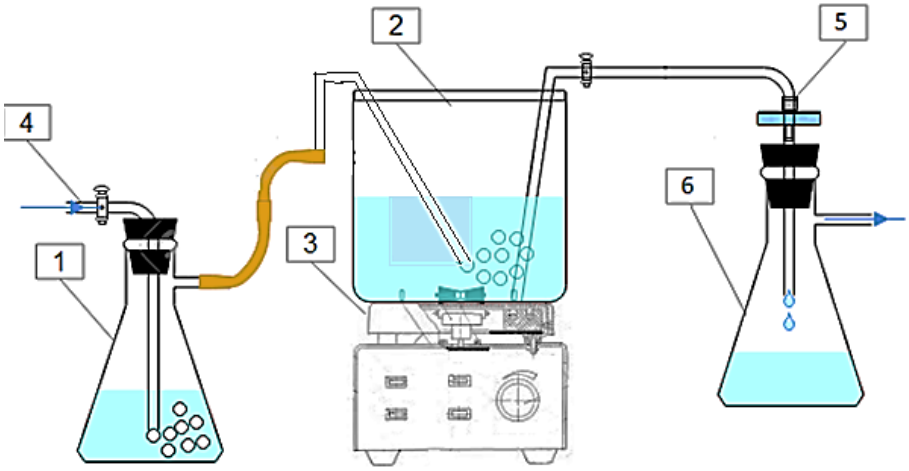

Fig. 1. Reaction apparatus for the preparation of the HMPAO-Sn(II) radiopharmaceutical kit.

A solution of HMPAO containing sodium chloride and mannitol is placed in a beaker on a magnetic stirrer. Inert gas argon, previously purified from oxygen, is passed through the solution for 10 minutes at a flow rate of 5×10^{-6} m³/s. Then, 2.5 ml of stannous chloride solution is pipetted from a flask and added to the beaker, followed by stirring. Inert gas is again passed through the solution for 10 minutes. Afterward, the pH of the final solution is adjusted to 9.4–9.8 by adding 1M NaOH solution. The resulting complex solution is filtered through a membrane filter with a pore size of 0.22 μm and aliquoted in 1.0 ml portions into drug vials. The vials are then subjected to lyophilization at a temperature range of -50°C to +25°C and under a vacuum of 0.36 Pa for 20.5 hours. Upon completion of the lyophilization process, the lyophilizer chamber is disconnected from the vacuum pump and filled with dry inert gas, which is passed through a column containing a desiccant ($CaCl_2$). After the chamber is filled with gas, the trays with vials are removed, the vials are sealed with rubber stoppers and aluminum caps, and hermetically sealed using a cap crimping device.

IV.METHOD OF OBTAINING THE RADIOPHARMACEUTICAL HMPAO^{99m}Tc.

To obtain the HMPAO^{99m}Tc complex, 3 ml of sodium pertechnetate solution, 99mTc, from the generator is introduced into a vial containing the lyophilized HMPAO-Sn(II) using a syringe, by piercing the rubber stopper with a needle. The sodium pertechnetate solution, 99mTc, must be obtained no later than 24 hours after the previous elution from the generator. After adding the sodium pertechnetate solution, 99mTc, the vial is shaken until the lyophilizate HMPAO-Sn(II) is completely dissolved. The efficiency of HMPAO^{99m}Tc complex formation is monitored. The amount of formed HMPAO99mTc complex, as well as unbound $99mTcO_4^-$ and hydrolyzed $99mTcO_2$, is determined using paper and thin-layer chromatography methods.

V. METHOD OF DETERMINING THE STABILITY OF THE KIT HMPAO-Sn(II).

The method for determining the stability of the kit HMPAO-Sn(II) is as follows: to study the stability of the kit HMPAO-Sn(II), previously prepared kits with a shelf life of 3, 6, 9 and 12 months were selected. The criterion for the stability of the kit HMPAO-Sn(II) is the preservation of its quality, i.e. appearance, quantitative content of basic substances and efficiency of labeling with radionuclide ^{99m}Tc.

VI. METHOD OF DETERMINING THE CONTENT OF COMPONENTS OF THE KIT HMPAO-Sn(II).

Determination of d,l-hexamethylpropyleneamine oxime (HMPAO)

The content of the ligand d,l-hexamethylpropylene-amine-oxime (HMPAO) was determined using a spectrophotometric method. Spectrophotometry allows for the measurement of light absorption by a substance in solution at a specific wavelength. In this case, a wavelength of 496 nm was chosen, which was experimentally determined to correspond to

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the maximum absorption for this ligand. The ligand content was calculated based on the measured optical density at the specified wavelength range.

Determination of Stannous Chloride ($SnCl_2$)

The content of Stannous Chloride ($SnCl_2$) was determined using a spectrophotometric method. Spectrophotometry allows for the measurement of light absorption by a substance in solution at a specific wavelength. In this case, a wavelength of 353 ± 2 nm was chosen, which was experimentally determined to correspond to the maximum absorption for Stannous Chloride ($SnCl_2$). The content of Stannous Chloride ($SnCl_2$) was calculated based on the measured optical density within the specified wavelength range.

VII. METHOD OF DETERMINING THE RADIOCHEMICAL PURITY OF HMPAO^{99m}Tc.

To study the radiochemical purity and content of radiochemical impurities, the work used the methods of thin-layer chromatography (TLC) and paper chromatography, which made it possible to determine the radiochemical purity and content of impurities in the HMPAO^{99m}Tc preparation with high accuracy.

VIII. DETERMINATION OF THE FUNCTIONAL SUITABILITY OF THE RADIOPHARMACEUTICAL HMPAO ^{99M}TC, PREPARED FROM THE HMPAO-SN(II) KIT, FOR SCINTIGRAPHIC DETECTION OF BRAIN PATHOLOGIES.

The study of the functional suitability of the radiopharmaceutical agent HMPAO^{99m}Tc, prepared from the HMPAO-Sn(II) kit, for scintigraphic detection of brain pathology was conducted using male Wistar rats weighing 300-350 g. Each experimental series involved 5 animals.

Prior to the procedures, the animals were anesthetized with ether to minimize stress and ensure safety during the manipulations. The radiopharmaceutical agent HMPAO^{99m}Tc, prepared from the HMPAO-Sn(II) kit, was then intravenously administered at a dose of 1.85 MBq/kg body weight through the femoral vein.

IX. RESULTS AND DISCUSSION.

In the study of the influence of ligand concentration, Sn^{2+} ions, and pH on the efficiency of HMPAO^{99m}Tc complex formation in solutions, HMPAO-Sn(II) complexes were synthesized with varying ligand concentrations ranging from 0.35 to 0.6 mg/ml, Sn^{2+} ion concentrations from 0.35 to 0.7 µg/ml, and pH values between 9.0 and 10.0. The lyophilized HMPAO-Sn(II) samples were dissolved by adding 3.0 ml of sodium pertechnetate solution ($Na^{99m}TcO_4$) obtained from a Technetium-99m generator. Immediately after dissolving the lyophilized samples, the amount of the formed HMPAO^{99m}Tc complex was determined, and the concentrations of unbound pertechnetate $^{99m}TcO_4^-$ and hydrolyzed 99mTcO_2 were measured using paper and thin-layer chromatography methods. The results of the study are presented in Table 1.

Table 1. Efficiency of formation of the HMPAO ^{99m}Tc complex depending on the concentration of HMPAO, Sn^{2+} and the pH of the complex solution (in percent), 20 minutes after adding $^{99m}TcO_4^-$ to the kit HMPAO -Sn(II).

HMPAO **mg/ml**	**Content of Sn^{2+}, µg /ml**								pH, solution complex
	0,35	0,4	0,45	0,5	0,55	0,6	0,65	0,7	
0,35	82,3	82,7	85,1	86,2	85,9	83,2	81,6	79,4	9,0
	82,6	83,1	85,6	86,9	86,1	84,2	82,1	81,7	9,5
	82,9	82,6	85,2	86,3	85,7	83,3	81,9	80,5	10,0
0,40	83,2	83,3	85,6	86,9	86,1	84,2	82,1	80,4	9,0
	83,6	85,7	87,1	88,2	87,6	84,9	83,7	80,7	9,5
	82,5	82,6	85,2	86,3	85,7	83,3	81,9	80,2	10,0
0,45	84,2	84,8	87,9	88,2	88,3	88,0	85,8	83,4	9,0
	85,1	85,4	88,4	90,9	88,7	88,2	87,0	86,7	9,5

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	84,0	85,2	88,2	89,8	88,3	87,1	85,9	84,2	10,0
0,55	83,2	83,3	84,7	87,2	85,1	84,9	83,8	80,4	9,0
	83,3	83,7	85,7	88,1	87,1	85,6	84,5	80,7	9,5
	82,1	82,9	84,6	86,3	85,2	84,7	82,9	79,2	10,0
0,60	82,0	82,1	84,9	87,1	85,3	83,1	82,1	80,0	9,0
	82,9	83,9	85,6	88,1	86,9	85,0	83,3	80,5	9,5
	82,0	82,5	84,2	86,0	85,0	83,1	80,9	78,2	10,0
	Efficiency of formation of the HMPAO ^{99m}Tc complex, %								

The results presented in the table indicate that the radiopharmaceutical kit with an HMPAO concentration in the range of 0.4–0.45 mg/ml, Sn^{2+} concentration of 0.5 µg/ml, and solution pH of 9.5 forms a relatively stable complex with 99mTc, with a complex formation efficiency exceeding 90.0%. Increasing the Sn^{2+} concentration to 0.6 µg/ml

in the reaction mixtures did not have a significant impact on the efficiency of HMPAO^{99m}Tc complex formation. However, further increasing the Sn^{2+} concentration in the reaction mixture to 0.7 µg/ml resulted in a noticeable decrease in complex formation efficiency. This reduction is presumably due to the excess Sn^{2+} ions promoting the reduction of $^{99m}TcO_4^-$ pertechnetate to a lower oxidation state of technetium, which interferes with the formation of a stable complex. The impact of the elevated Sn^{2+} concentration on the reduction of $^{99m}TcO_4^-$ may indicate competition between the reduction and complexation processes, leading to a decrease in the yield of the HMPAO^{99m}Tc complex.

The kinetics of complex formation and stability of the HMPAO^{99m}Tc complex were studied over different time intervals. The starting components consisted of the HMPAO - Sn(II) kit, containing Sn2+ at a concentration of 0.5 µg/mL, HMPAO at 0.45 mg/mL, and a solution pH of 9.5. To form the complex, 5.0 mL of sodium pertechnetate solution (Na^{99m}TcO4) was added.

Chromatographic analysis was conducted at various time points: 0 min, 5 min, 10 min, 20 min, 25 min, 30 min, 1 h, 3 h, 5 h, and 6 h. Paper and thin-layer chromatography methods were used to determine the composition of the preparation.

The results of the study indicated that immediately after the formation of the complex, the HMPAO^{99m}Tc complex contained no less than 90% of the complex. Furthermore, the stability of the complex remained high for at least 2.5 hours, as confirmed by the chromatographic data (Fig. 2).

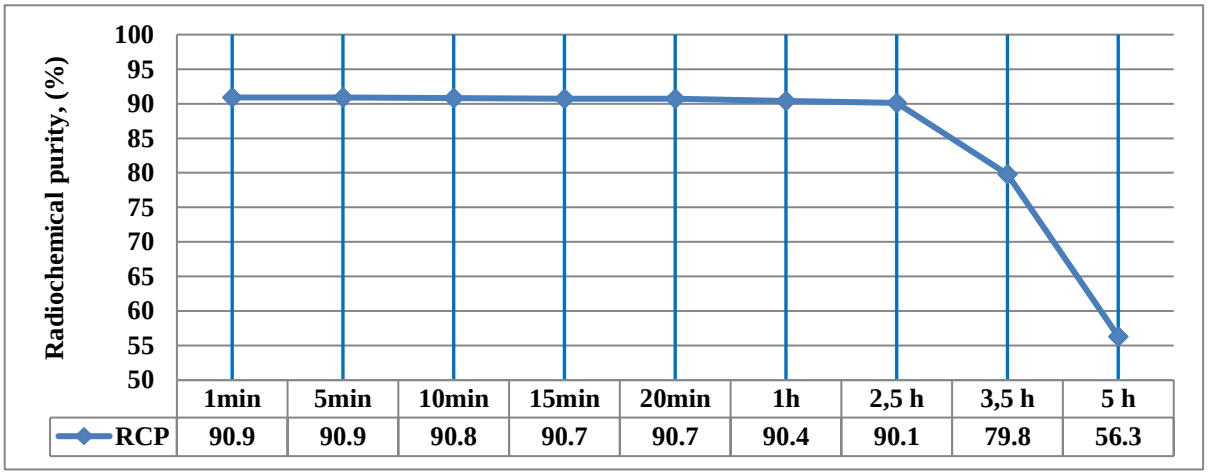

Fig. 2. Kinetics of complex formation and stability of the HMPAO^{99m}Tc complex at various time intervals

To assess the stability of the HMPAO-Sn(II) complex, vials containing the lyophilizate were stored at 2-8°C for a period of 13 months. During this period, regular stability studies were conducted, including the measurement of radiochemical purity (RCP) and the control of active component content.

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The results of the analysis are presented in Table 2, which shows data on the concentration and stability of the formed radiopharmaceutical complexes.

Table 2. Stability studies of kit HMPAO-Sn(II)

The name of indicators	Data on the day of production	Time after production 3 months	Time after production 6 months	Time after production 9 months	Time after production 12 months	Time after production 13 months
Description	white lyophilisate	white lyophilisate	white lyophilisate	white lyophilisate	white lyophilisate	white lyophilisate
Content of Sn^{2+} , µg /ml	0,5 ±0,02	0,49 ±0,02	0,5 ±0,02	0, 48 ±0,02	0,48 ±0,02	0,41 ±0,02
Content of HMPAO, mg/ml	0,45 ±0,02	0,45 ±0,02	0,44 ±0,02	0,45 ±0,02	0,43 ±0,02	0,39 ±0,02
RCP HMPAO^{99м}Tc, в %	90,5±0,2	90,3±0,2	90,4±0,2	90,2±0,2	90,1±0,2	78,7±0,2

Based on the provided data, the radiochemical purity (RCP) of HMPAO^{99m}Tc obtained from lyophilized kits, stored at 2-8°C for 12 months, was 90.1 ± 0.2%. This value is slightly lower than the RCP measured on the production day; however, the difference in the readings is not significant and falls within acceptable limits. As a result, based on these findings, the shelf life of the lyophilized kits for preparing individual doses of HMPAO^{99m}Tc has been established as 12 months when stored within the temperature range of 2-8°C.

A spectrophotometric method was used for the quantitative determination of the active ingredient, d,l-hexamethylpropylamine oxime, in solutions of the HMPAO-Sn(II) kit. In the UV absorption spectrum of d,l-hexamethylpropylamine oxime solutions, a peak is absorbed at 496 nm within the wavelength range of 470 to 510 nm (Table3). The selectivity of this analytical method was confirmed by comparing the placebo solution with the HMPAO-Sn(II) solution. Spectral analysis of placebo solutions and HMPAO-Sn(II) solutions revealed that the excipients do not affect the position of the absorption maxima of d,l-hexamethylpropylamine oxime, although they exhibit a small intrinsic absorption in the investigated wavelength range(Figure3).

Table 3. Measurement results solutions of placebo and HMPAO-Sn(II)

Wavelength, nm	Optical density of placebo solution	Optical density of solution HMPAO-Sn(II)
465	0,01	0,286
470	0,012	0,286
475	0,015	0,286
480	0,021	0,291
485	0,03	0,312
490	0,033	0,327
496	0,033	0,338
498	0,024	0,329
500	0,025	0,321
505	0,022	0,306
510	0,021	0,289

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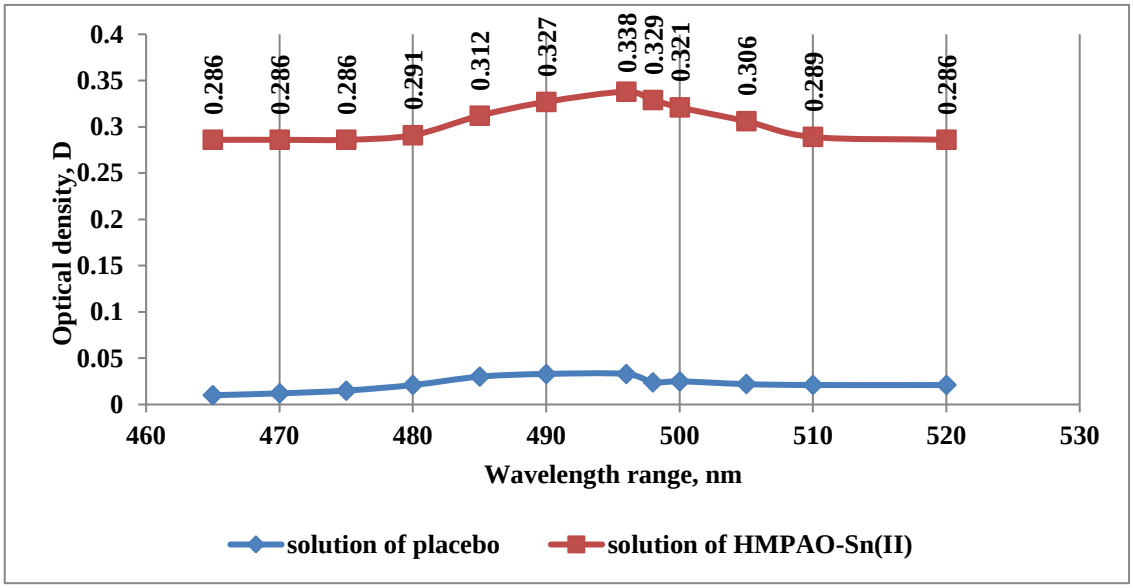

Fig 3 Optical density curve solutions of HMPAO -Sn(II) and placebo.

The content of d,l-hexamethylpropylamine oxime (d,l-HMPAO) in the solution is determined based on the measured optical density. To do this, 2.0 ml of an acetate buffer solution with a pH of 5.8–6.0 is placed into a 5 ml test tube, then 1.3 ml of a 0.005 M copper chloride solution and 1.7 ml of the lyophilized solution are added, and the solution is mixed. A colored complex of d,l-HMPAO with Cu(II) is formed, allowing the measurement of the optical density of the solution using a spectrophotometer at a wavelength of 496 nm in a 2 cm path length cuvette.

For the comparison solution, all components are used except the lyophilized solution, which is replaced by 1.7 ml of 0.9% sodium chloride solution for injections.
The content of d,l-HMPAO in the solution is calculated using the following formula:

$$G_{\text{d,l -HMPAO}} = \frac{C \cdot 3{,}0 \cdot 5{,}0}{1{,}7},$$

Where:

- C_x - concentration of d,l-HMPAO in the test solution, determined using the calibration curve, in milligrams per milliliter (mg/ml);
- 3.0 - volume of the lyophilized solution, in milliliters (ml);
- 5.0 - volume of the test solution, in milliliters (ml);
- 1.7 - volume of the lyophilized solution, in milliliters (ml).

To construct the calibration curve, approximately 0.05 g of d,l-HMPAO, previously dried to a constant mass at a temperature of 100-105 °C, is dissolved in a 50 ml volumetric flask with a 0.9% sodium chloride solution (ultrasonic bath can be used to accelerate dissolution). The volume is then adjusted to the mark with the 0.9% sodium chloride solution.
In six 25 ml volumetric flasks, prepare the reference solutions by adding 1.5, 3.0, 6.0, 9.0, 12.0, and 15.0 ml of the stock solution to each flask, then adjust the volume to the mark with the 0.9% sodium chloride solution and mix thoroughly.
In each of six 5 ml test tubes, add 2.0 ml of acetate buffer solution, 1.3 ml of 0.005 M copper chloride solution, and 1.7 ml of the prepared reference solutions, then mix. Measure the optical density of the resulting solutions using a spectrophotometer at a wavelength of 496 nm in 10 mm pathlength cuvettes. For the reference solution, use a mixture

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containing all components except for the reference solution, substituting it with 1.7 ml of the 0.9% sodium chloride solution.
The calibration curve is plotted with optical density on the y-axis and d,l-HMPAO concentration mg/ml on the x-axis (Figure4).

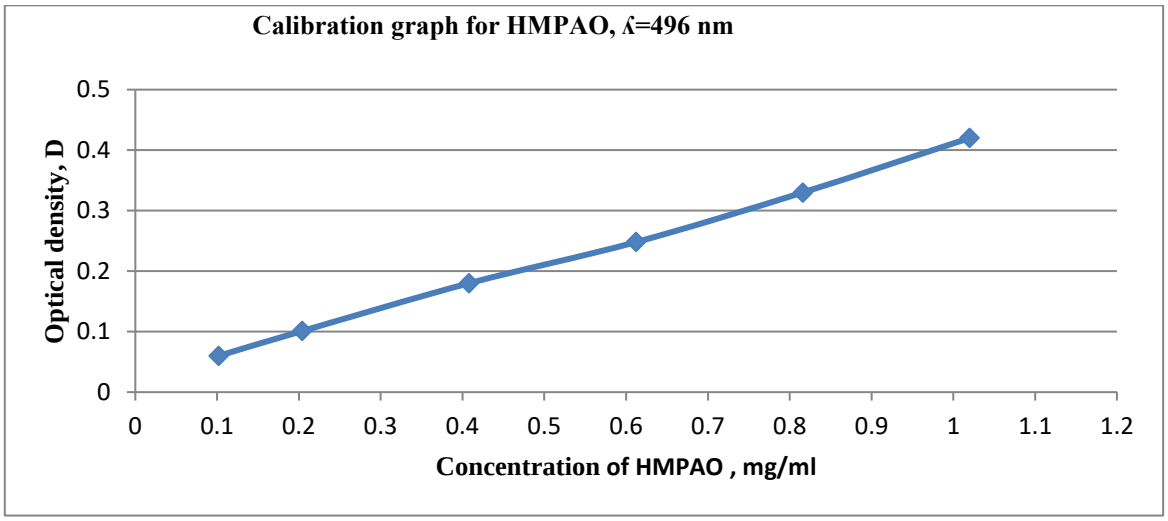

Figure 4. Calibration graph of the dependence of the change in optical density of standard solutions on the concentration of HMPAO.

The method for determining the concentration of tin(II) chloride involves spectrophotometric analysis of a solution containing 4.5 ml of 2 M hydrochloric acid, 0.3 ml of 0.16% potassium perrhenate solution, 0.1 ml of 20% potassium thiocyanate solution, and 0.1 ml of the lyophilizate solution. After mixing, the optical density of the resulting solution is measured at a wavelength of 353 nm in a 10 mm path length cuvette. As a comparison solution, a similar solution is used, but instead of the lyophilizate solution, 0.1 ml of a 0.9% sodium chloride isotonic solution for injection is added. The tin (II) concentration (C) in the test solution is calculated using the following formula:

$$G_{Sn^{2+}} = \frac{Д \cdot 118{,}69 \cdot 0{,}1 \cdot 4{,}6}{16800 \cdot 0{,}1} \times 1{,}61$$

where:

- D – optical density of the test solution;
- 4.6 – volume of the test solution in milliliters;
- 0.1 – volume of the reagent solution in milliliters;
- 118.69 – atomic mass of tin (Sn);
- 0.1 – volume of the reagent solution used for analysis, in milliliters;
- 1.61 – coefficient for calculating anhydrous stannous chloride ($SnCl_2$) from divalent tin (Sn^{2+});
- 16800 – conditional molar absorption coefficient (according to the data in "Practical Course in General Biochemistry" by Yu.B. Filippovich et al., Moscow, Prosveshchenie, 1982).

To study the radiochemical purity of HMPAO^{99m}Tc, thin-layer chromatography (TLC) and paper chromatography methods were used in the work.
The radiochemical purity of a radiopharmaceutical is the proportion of total radioactivity in the desired form. The HMPAO^{99m}Tc preparation may contain a radiochemical impurity, free pertechnetate ($^{99m}TcO_4^-$) and hydrolyzed reduced technetium. This component is characterized by the Rf value, which is defined as the ratio of the distance traveled by the component from the starting point of application of the compound under study.
A. A sample of HMPAO^{99m}Tc (0.001-0.005 ml) is applied to a chromatographic strip (10x100 mm) of silica gel (e.g., Art. 5553, Merck) or a similar type, which has been pre-prepared according to the manufacturer's instructions, with a 15 mm margin from the edge (starting line). The amount of the sample should be sufficient to statistically detect at least 0.5% of the applied activity on a radiometric device. Chromatography is immediately performed (without drying the

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spot) using the ascending method with methyl ethyl ketone as the mobile phase, until the front of the mobile phase reaches the upper edge of the strip (approximately 5-10 minutes). Under these chromatographic conditions, the Rf value for the HMPAO^{99m}Tc complex and $^{99m}TcO_4^-$ is 0.8-1.0 ± 0.05, while the Rf for hydrolyzed reduced $^{99m}TcO_2$ is 0.0-0.5. The resulting chromatogram is air-dried and then covered with polyethylene tape (with adhesive layer, on both sides. The counting rate is measured from the section containing pertechnetate ions ($^{99m}TcO_4^-$) and from the entire chromatogram using a radiometric method (Figure 5). Based on the obtained data, the relative content of pertechnetate ions (X1) in percentage is calculated using the following formula:

$$X1=N1/N2\times100$$

where:

- N1 is the counting rate in the upper section of the chromatogram containing technetium-99m ions, in counts per second (cps);
- N2 is the total counting rate for the entire chromatogram, in counts per second (cps).

B. The analysis of the radiochemical purity of the HMPAO^{99m}Tc preparation is performed using paper chromatography. For this, a strip of chromatography paper, grade Whatman 3MM, measuring 15 x 200 mm, is used, and a sample of the preparation, ranging from 0.001 to 0.005 ml, is applied to the starting line, located 15 mm from one edge. The strip is then subjected to ascending chromatography using a 0.9% sodium chloride solution as the solvent. The chromatography process continues until the solvent reaches the front line, located 100 mm from the starting line. The chromatography time is typically around 40 minutes.

After chromatography, the distance traveled by the various components of the preparation is determined. The Rf of the pertechnetate ions ($^{99m}TcO_4^-$) is typically 0.8-1.0, while the Rf of other components of the preparation, such as HMPAO^{99m}Tc and $^{99m}TcO_2$, ranges from 0.0 to 0.5. After the chromatogram is dried in the air and adhered on both sides with polyethylene tape with an adhesive layer, the radiometric activity is measured both from the area containing hydrolyzed reduced technetium (HRT) and from the entire chromatogram. To assess the radiochemical purity of the preparation, the relative content of HRT (**X2**) is calculated as a percentage, based on the measured radiometric activity (Figure 6). The radiochemical purity of the preparation (**X**) is then calculated using the following formula: X=100−(X1+X2),

where:

- X1 is the relative activity of pertechnetate ions in percentage;
- X2 is the relative activity of hydrolyzed reduced technetium in percentage.

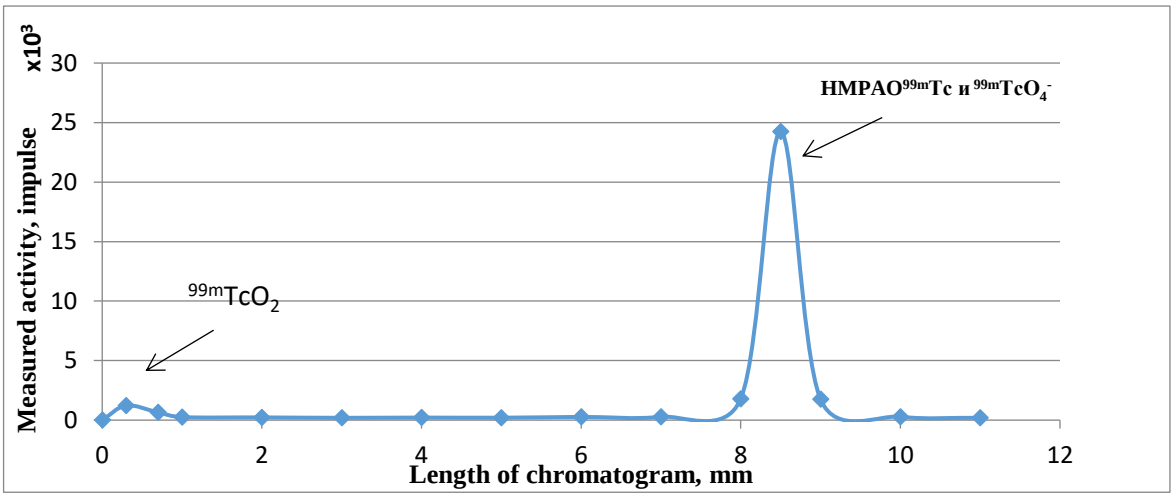

Fig. 5. Distribution of HMPAO^{99m}Tc according to the chromatogram (Solvent: methyl ethyl ketone)

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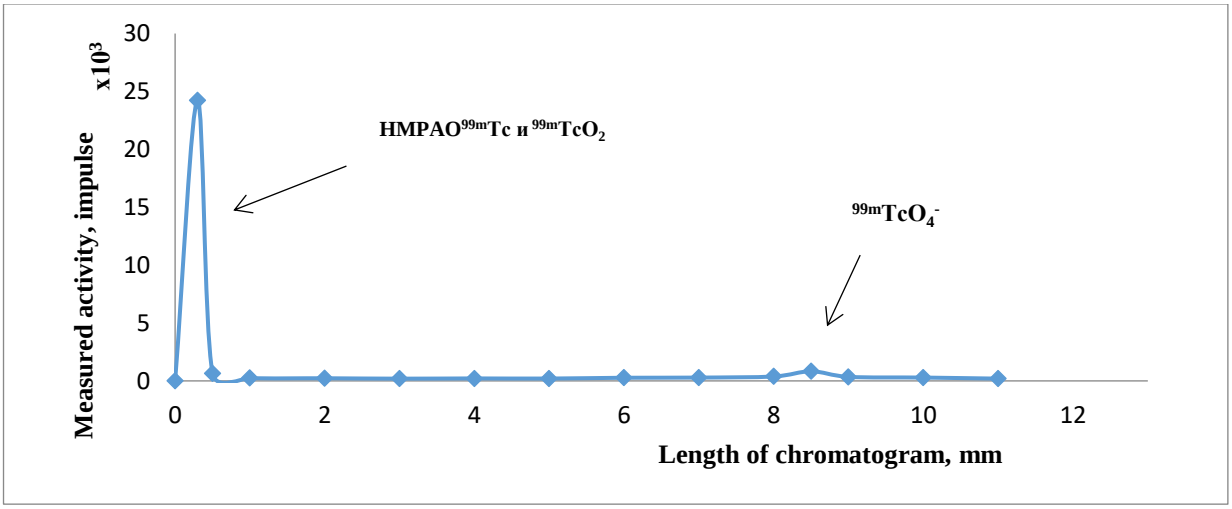

Fig. 6. Distribution of HMPAO^{99m}Tc according to the chromatogram (Solvent: 0.9% sodium chloride solution)

The test results presented in figure 4,5. prove the specificity of the method for determining the content of the radiochemical impurity pertechnetate ions $^{99m}TcO_4^-$and $^{99m}TcO_2$ in the HMPAO^{99m}Tc preparation.
Methods for qualitative and quantitative analysis of radiopharmaceutical kits have been developed. Based on the results obtained, a draft pharmacopoeial monograph of the enterprise (FSP) was prepared.
The study of the functional suitability of the radiopharmaceutical agent HMPAO^{99m}Tc was conducted on 30 white rats weighing 300-350 g, used to evaluate the pharmacokinetic properties of the drug. HMPAO^{99m}Tc was administered intravenously into the tail vein at a volume of 0.2 ml.
After the injection, the animals were sacrificed at various time intervals: 1, 5, 15, 30, 60 minutes, 2, 3, and 24 hours. The animals were decapitated, a method that allowed for the exclusion of stress and anesthesia's influence on pharmacokinetic processes such as distribution and elimination of the drug. The content of the radionuclide in organs and tissues was determined by direct radiometry using an automatic spectrometer. This ensured accurate measurements of the concentration of HMPAO^{99m}Tc in various tissues of the body. The results were expressed as percentages of the administered dose, allowing the assessment of its distribution across organs and tissues (Table 4).
For pharmacokinetic analysis in blood, the concentration of the radionuclide was also determined, expressed as a percentage of the administered volume per gram of blood. The total circulating blood volume was considered to be 7% of the animal's body weight. All obtained data were used for further calculations aimed at assessing the distribution dynamics of the drug in the body over different time intervals.

Table 4. Concentrations of HMPAO^{99m}Tc radioactivity in some organs and tissues after injection from 1 minute to 24 hours in % of the administered dose

Organs	Minutes					Hours		
	1	5	15	30	60	2	3	24
Brain	2,8±0,1	2,78±0,08	2,8±0,09	2,78±0,05	2,80±0,1	2,65±0,07	2,50±0,1	1,30±0,1
Blood	19±1,2	16,5±1,2	16,0±1,1	16,5±1,2	16,2±1,0	15,5±1,3	14,0±1,0	7,3±1,0
Skeletal muscles	39,5±1,2		34,5±2,3	27,5±1,2	28,7±2,1	25,5±2,0	20,7±2,2	12,6±2,0
Skeleton	3,2±0,1	2,8±0,08	3,2±0,07	2,9±0,1	2,5±0,07	3,0±0,08	2,8±0,1	2,9±0,0
Lungs and heart	6,0±0,3	6,5±0,2	6,3±0,2	6,3±0,3	6,0±0,1	4,3±0,1	3,0±0,1	2,0±0,1
Liver and spleen	9,5±0,5	8,5±0,3	8,3±0,4	7,0±0,2	6,5±0,4	6,0±0,4	5,0±0,3	2,5±0,3
Stomach					0,7±0,05	0,8±0,04	0,7±0,05	0,5±0,1

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Small and large intestines	13,0±1,5	12,9±1,5	13,2±1,6	13,0±1,7	13,5±1,8	18,0±0,2	23,2±2,7	34,5±3,0
Kidneys	5,2±0,9	4,8±0,8	5,0±0,6	5,8±0,5	5,5±0,7	4,5±0,6	4,0±0,9	3,1±0,1
Urine excretion	-	4,5±0,3	5,0±0,5	14,0±1,2	16,0±1,5	-	-	
Urine and feces excretion	-	4,5±0,3	5,0±0,5	14,0±1,2	16,0±1,5	18,5±0,2	20,0±2,5	30,0±2,0

Based on the data in Table 4, it is evident that within the first minutes after administration, the radiopharmaceutical HMPAO^{99m}Tc is rapidly distributed and absorbed by the brain, establishing a stable binding with carriers—specific proteins and biostructures of the tissue. This confirms the high affinity of the compound to the corresponding molecular structures and brain tissues during the early period post-administration.

Over the first hour after administration, technetium-99m remains strongly bound to the brain tissue, which accounts for its minimal clearance during this period. The first signs of isotope clearance from the bloodstream are observed after two hours, indicating the gradual onset of elimination processes from the organism.

The half-life of technetium-99m is approximately 24 hours, which suggests an extended period of circulation in the body until its equilibrium concentration in the bloodstream is achieved.

It is important to note that the clearance rate of technetium-99m from the body does not directly correlate with the strength of its binding to the brain tissue structures. This highlights the complexity of the biokinetic processes occurring in the body, where tissue binding does not always directly correlate with elimination dynamics.

X. CONCLUSION

Thus, in the course of the research work, the optimal ratios of the main substance HMPAO and the reducing agent (Sn2+) in the HMPAO^{99m}Tc complexation reaction were determined, which amounted to 0.4–0.45 mg/ml and 0.5 μg/ml, respectively, created developed technology for obtaining of the kit HMPAO-Sn(II). The 5 batches of the HMPAO -Sn(II) kit produced have a radiochemical purity of more than 90.0%, the shelf life of the kit HMPAO-Sn(II) was determined, which was 12 months, and animal tests showed that the radiopharmaceuticals of HMPAO^{99m}Tc is rapidly distributed and absorbed by the brain, establishing a stable binding with carriers—specific proteins and biostructures of the tissue, allows you to identify brain tissues pathology during the early period post-administration with high reliability.

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