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Preparation and Study of Stability Pharmaceutical Composition [PSMA-11] and Radiopharmaceutical [^{99m}tc]-PSMA-11 Labeled With ^{99m}tc Radionuclide

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ABSTRACT

This work is devoted to the study of the synthesis of a pharmaceutical composition (PhC) [PSMA-11] in the form of a lyophilisate with optimization of the amount of ingredients and sodium pertechnetate ($Na^{99m}TcO_4$) from the ^{99m}Tc Generator to obtain the radiopharmaceutical [^{99m}Tc]-PSMA-11 with the highest possible radiochemical yield (RCY) and radiochemical purity (RCP). The stability of radiopharmaceutical [^{99m}Tc]-PSMA-11 in 0.9% NaCl solution and in human serum was determined. The preliminary shelf life of PhC [PSMA-11] was determined and set to 6 months.

I. INTRODUCTION

In recent decades, the most common malignant neoplasm among men is prostate cancer, which on average accounts for 20% of all cases of malignant neoplasms [1]. In this regard, positron emission tomography (PET) with targeted prostate-specific membrane antigen (PSMA) has become an essential part of prostate cancer (PCa) imaging. According to [2], in some national guidelines, this method is preferred for detecting a lesion in biochemical recurrence (BCR) after primary treatment and is mandatory before radionuclide therapy using PSMA. However, in many countries, access to patients in need of PET examinations is often limited, either because of the high cost of PET procedures or because of the limited availability of nuclear centers. In this regard, one of the important clinical problems of modern medicine is the early detection and visualization of recurrences after prostatectomy, primary and metastatic prostate cancer in all categories of patients, which will subsequently help timely decision-making on the exact targeted therapy of this disease. For this purpose, an expensive ^{68}Ga-HBED-CC-PSMA (PSMA-11) PET/Computer Tomography (PET/CT) diagnostic method is currently used in clinical settings, which is not available to the general population. In recent studies, it has been demonstrated that the ^{99m}Tc-labeled PSMA inhibitor [^{99m}Tc]Tc-MIP1404, which allows SPECT scanning, detects PSMA-positive lesions with high sensitivity in patients with biochemical recurrence of PCa (70% and 77% of those examined, respectively) [3-4].

Thus, based on economic considerations, it is required to develop a technology for obtaining a radiopharmaceutical based on the indicator ligand PSMA-11 labeled with the ^{99m}Tc radionuclide, which allows registration on SPECT/CT to provide inexpensive medical support to all segments of the population, especially those in need of social protection of patients.

The present work is devoted to the synthesis of a pharmaceutical composition with the PSMA-11 ligand peptide and the radiopharmaceutical [^{99m}Tc]-PSMA-11 with the highest possible radiochemical yield (RCY) and radiochemical purity (RCP). Also, the study of the stability of the synthesized radiopharmaceutical in 0.9% NaCI solution and human serum and the determination of the shelf life of pharmaceutical composition (PhC) [PSMA-11].

The synthesized radiopharmaceutical [^{99m}Tc]-PSMA-11 is subsequently used as a diagnostic tool for SPECT-CT of positive PSMA antigen in men with prostate cancer, with suspected metastases, with suspected recurrence based on

an elevated level of prostate-specific antigen (PSA) in human blood serum.

II. EXPERIMENTAL Materials.

All reagents and laboratory reagents used in the work were of the highest purity (unless otherwise indicated). The PSMA-11 inhibitor was purchased from MedChemExpress. Stannous chloride dihydrate was purchased from Sigma Aldrich. Solvents and reagents were purchased from Sigma Aldrich unless otherwise noted and were used without further purification. Determination of the RCY of the labeling process was carried out by determining the RCP of radiopharmaceutical [^{99m}Tc]-PSMA-11. When determining the RCY of the labeling process, a mixture of solvents acetonitrile and chemically pure ethyl alcohol was used as the mobile phase. Human serum for stability studies of the PhC of [PSMA-11] and radiopharmaceutical [^{99m}Tc]-PSMA-11] was obtained from OOO Blood Products (Republican Center for Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan).

Equipment

All radiometric measurements were carried out on a four-channel gamma spectrometer NP-424L (Hungary) and Ludlum 2200 (USA). The scintillator used in the complex is a NaI(Tl) crystal, which makes it possible to increase the measurement geometry up to 4π. Activity measurement range 50-104 Bq and energies 20-3000 KeV. Measurements of quantitative and qualitative activities of radionuclides were carried out on a gamma spectrometric device ASPECT SU-03P with a semiconductor Ge(Li) detector. Radionuclides were identified by their gamma lines. A Mettler Toledo Seven Easy pH meter (Switzerland) was used to measure and correct the pH of the analyzed solutions of preparations and buffer solutions. Solutions were filtered using membrane filters with a pore size of 0,22 μm manufactured by Millipore Express (PES). Lyophilization of reagent solutions of the PhC PSMA-11 was carried out in a freeze-dryer «Epsilon 2-16D».

Labeling of PSMA-11 with technetium-99m was carried out in a STEGLER WB-4 water thermostat-bath. Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from the «Generator of ^{99m}Tc» manufactured by the State Enterprise "Radiopreparat" with a nominal activity of 18,5 GBq. RCP and RCY of $Na^{99m}TcO_4$ with PSMA-11 were evaluated by thin layer chromatography (TLC). TLC Kieselgel 60 thin-layer plates (TLP) with a thin layer of Merck silica gel (DC-Alufolein) were used as the TLC stationary phase.

III. RESULTS AND DISCUSSION

Synthesis of batches of Pharmaceutical Composition [PSMA-11]

To synthesize the reagent solution of the PhC of [PSMA-11], an aqueous solution of peptide-ligand of PSMA-11 with a content of 15–50 μg/ml was introduced into a solution of an ascorbate buffer solution with concentration of 25–150 mg/ml and pH 5, sodium tartrate and L-cysteine with a content of 10–30 and 0,2–1,6 mg/ml, respectively. The contents of the ingredients of the reaction mixture given are those of the final solution. After complete dissolution of all ingredients, a hydrochloric acid solution of tin dichloride was added. The content of $SnCI_2$ in the reaction mixture was 40 μg/ml. The process of synthesizing a solution of a parmaceutical composition was carried out by passing an inert gas through the solution of the reaction mixture. Then the solution of the parmaceutical compositionwas passed through a membrane filter with a pore size of 0,22 μm, dispeced in vials for medicines and frozen at a temperature of -50 ^{0}C, followed by lyophilization for 7 hours with a temperature gradient from -50 ^{0}C, increasing the temperature by 5 ^{0}C every 30 min to + 15 ±2 ^{0}C. To obtain the [^{99m}Tc]-PSMA-11 radio pharmaceutical, the PhC [PSMA-11] was dissolved in a solution of sodium pertechnetate from the «Generator, Tc-99m» with an activity of 37-2500 MBq, after which it was incubated in a water thermostat-bath at a temperature of 90 ^{0}C for 20 minutes.

The determination of the radiochemical yield of the process of labeling the lyophilizate of Pharmaceuticals Composition of [PSMA-11] was carried out by determining the RCP of the radiopharmaceutical [^{99m}Tc]-PSMA-11 by the TLC method described in [5].

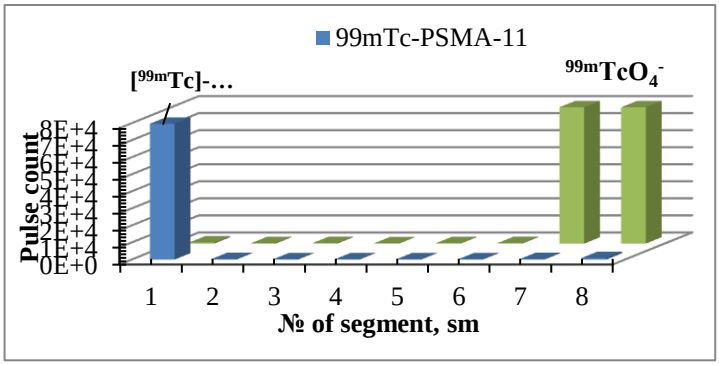

Figure 1. Distribution of the radiopharmaceutical [^{99m}Tc]-PSMA-11 on TLC "TLC Kieselgel 60", MF - CH_3CN:C_2H_5OH, n=6.

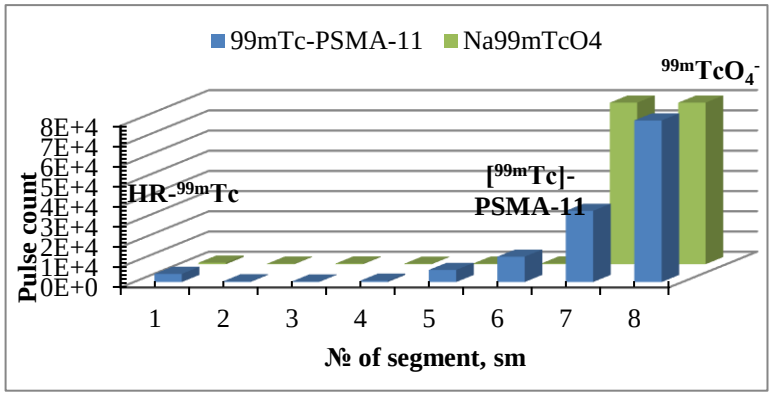

Figure 2. Distribution of the radiopharmaceutical [^{99m}Tc]-PSMA-11 on TLP «TLC Kieselgel 60», MF - 1M CH_3COONa:CH_3OH, n=6.

The obtained results of measurements of the radiochemical purity of the synthesized of the radiopharmaceutical [^{99m}Tc]-PSMA-11 showed that the radiochemical yield of labeling was more than 98.5±0.5% at n=6.

The results of the synthesis of the PhC [PSMA-11] and the radiopharmaceutical [^{99m}Tc]-PSMA-11 with various amounts peptide-ligand of the PSMA-11 and ascorbic acid are shown in Figures 3 and 4. The labeling process was carried out by introducing Na$^{99m}TcO_4$ with an activity of 1,85 GBq from a ^{99m}Tc generator.

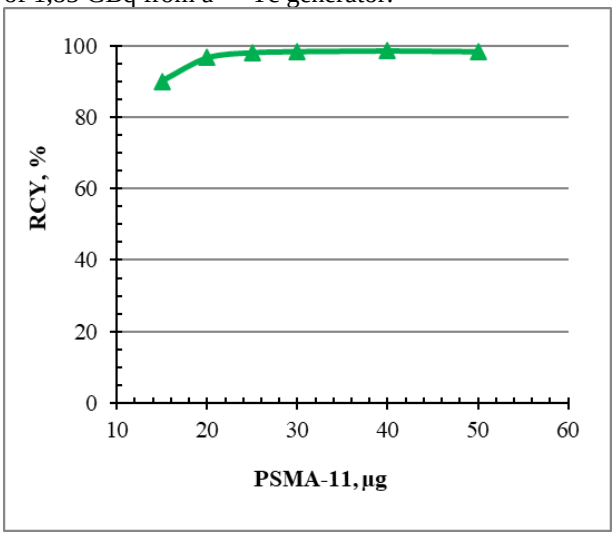

FIGURE 3. The influence of the amount of PSMA-11 on the RCY of labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6.

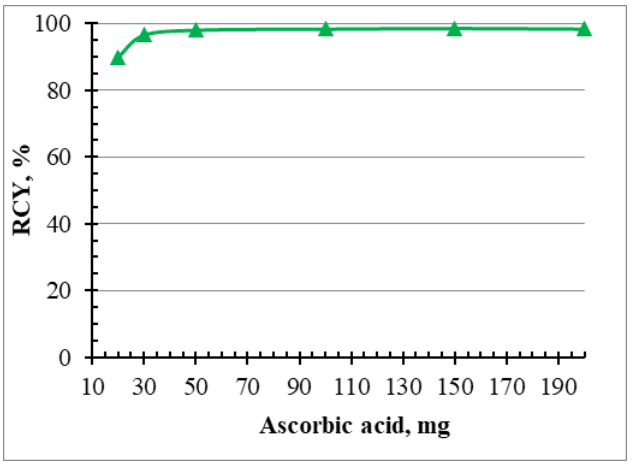

FIGURE 4. The influence of the amount of Ascorbic acid on the RCY of labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6.

As can be seen from Figures 3 and 4, in the synthesis of PhC lyophilisate with a content of the peptide-ligand of PSMA-11 less than 25 µg, the RCY of labeling is low, and at more than 25 µg, it remains stably maximum, and in the case of ascorbic acid, a high percentage of RCY of labeling is observed at the amount of ascorbic acid 50 mg or more. Labeling of the lyophilisate of PhC [PSMA-11] with ^{99m}Tc with the amount of peptide-ligand of PSMA-11 less than 20 µg promoted the formation of hydrolyzed ^{99m}Tc, and this led to a decrease in RCY labeling. Studies of the effect of ascorbic acid on RCY labeling showed that the amount of ascorbic acid below 50 mg/ml in the RCY labeling reaction mixture was below 90%, and this may be due to insufficient capacity of the buffered system.

For the synthesis of radiopharmaceutical [^{99m}Tc]-PSMA-11 with a consistently high RCP, we studied the effect of sodium tartrate and L-cysteine on RCY labeling. For this purpose, lyophilizates of PhC [PSMA-11] were synthesized with various amounts of sodium tartrate and L-cysteine ingredients, followed by the synthesis of the [^{99m}Tc]-PSMA-11 radiopharmaceutical. The research results are shown in Figures 5 and 6.

From the results of the studies (Fig. 5) it can be seen that in the synthesis of the lyophilizate PhC [PSMA-11] without including sodium tartrate ($C_4H_4Na_2O_6x2H_2O$) in the set the RCY of [^{99m}Tc]-PSMA-11 labeling is at the level of 70.2±1.9%, after the inclusion of the sodium tartrate ingredient in the composition of PhC [PSMA-11] in an amount of 10 mg/vial, the value of RHY labeling [^{99m}Tc]-PSMA-11 increased by 10%, and with an increase in the content of the sodium tartrate ingredient to 20 mg/vial, the RCY labeling of [^{99m}Tc]-PSMA-11 increased to 99.0%.

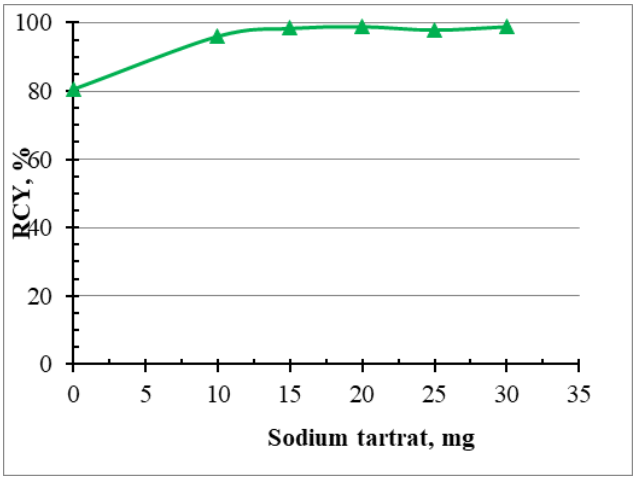

FIGURE 5. Influence of the amount of C4H4Na2O6 on the RCY labeling of radiopharmaceuticals [99mTc]-PSMA-11, n=6.

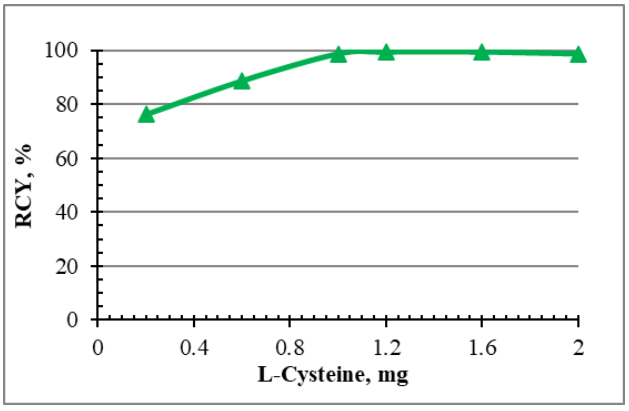

FIGURE 6. Influence of the amount of L-Cysteine on the RCY labeling of radiopharmaceuticals [99mTc]PSMA-11, n=6.

Also, the inclusion of another ingredient L-cysteine (Fig. 6) in the composition of PhC of [PSMA-11] in an amount of 1.2 mg/vial led to a stable high RCY labeling of [^{99m}Tc]-PSMA-11. This is explained by the fact that, apparently, the presence of L-cysteine in the composition of the lyophilizate of PhC of [PSMA-11] in combination with sodium tartrate prevents the oxidation of divalent tin during the synthesis by forming a complex with divalent tin.

Stability study of radiopharmaceutical [^{99m}Tc]-PSMA-11 and PhC of [PSMA-11].

Study of the stability of the radiopharmaceutical [^{99m}Tc]-PSMA-11 was carried out by synthesizing radiopharmaceutical [^{99m}Tc]-PSMA-11 in saline (0.9% NaCI solution) and kept them at room temperature for 24 hours. In the study of the stability of the radiopharmaceutical [^{99m}Tc]-PSMA-11 in human serum, the synthesized radiopharmaceutical [^{99m}Tc]-PSMA-11 in a volume of 0.2 ml was injected with 1.8 ml of human serum and kept at room temperature for 24 hours, and the stability of the radiopharmaceutical was determined by measuring RCP of the studied radiopharmaceutical samples after 2, 4, 8, 12, 16 and 24 hours.

The results of measuring the stability of radiopharmaceutical [^{99m}Tc]-PSMA-11 in 0.9% NaCl solution and in human serum are shown in FIGURES 7-8.

As can be seen from the figures, as a result of the series of measurements of the stability of the radiopharmaceuticals [^{99m}Tc]-PSMA-11 both in 0.9% NaCl solution and in human serum, it can be seen that the radiopharmaceutical [^{99m}Tc]-PSMA-11 is stable for more than 4 hours and the measured radiochemical purity of radiopharmaceuticals values were more than 98±0.5%. And in the case of further storage of radiopharmaceutical [^{99m}Tc]-PSMA-11 at room temperature for 8 hours to 27 hours, the radiochemical purity of the radiopharmaceutical [^{99m}Tc]-PSMA-11 decreased from 97±1.0% to 92±2.3%, respectively.

Thus, it can be concluded from this that the resulting radiopharmaceutical [^{99m}Tc]-PSMA-11 from PhC of [PSMA-11] is stable for 8 hours both in physiological solution and in human serum, which is sufficient for biological and clinical studies.

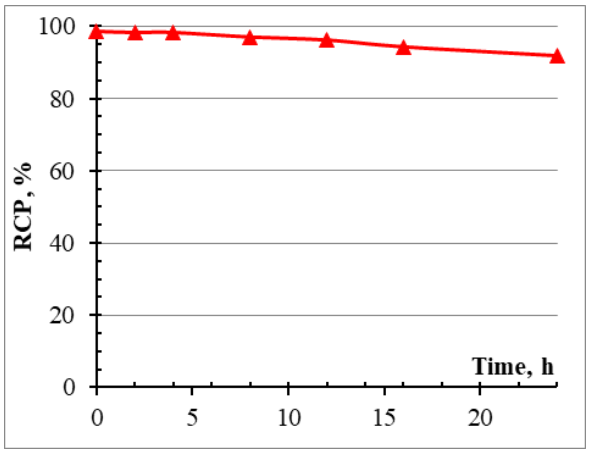

Figure 7. Graph of the stability of the radio-pharmaceutical [^{99m}Tc]-PSMA-11 in human serum.

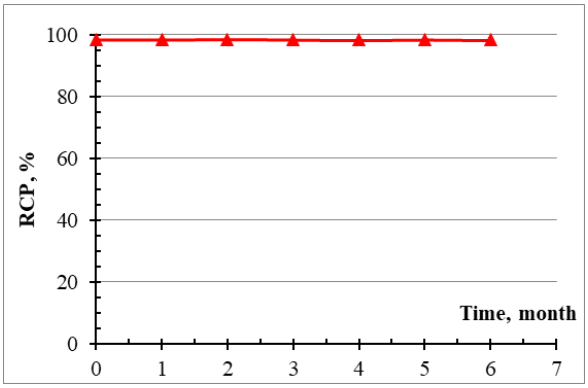

Figure 8. Graph of the stability of the radiopharma-ceutical [^{99m}Tc]-PSMA-11 in the 0,9% solution of NaCI.

In order to determine the expiration date of lyophilized PhC of [PSMA-11], vials with the PhC [PSMA-11] were stored at a temperature of 3-5 ^{0}C for 6 months and the stability of the PhC [PSMA-11] was studied by measuring radiochemical purity after the synthesis of radiopharmaceutical [^{99m}Tc]-PSMA -11 by the above method from stored PhC [PSMA-11] for 1 to 6 months.

The results of measurements of the RCP of radiopharmaceutical [^{99m}Tc]-PSMA-11 in determining the expiration date of PhC [PSMA-11] are shown in FIGURE 9.

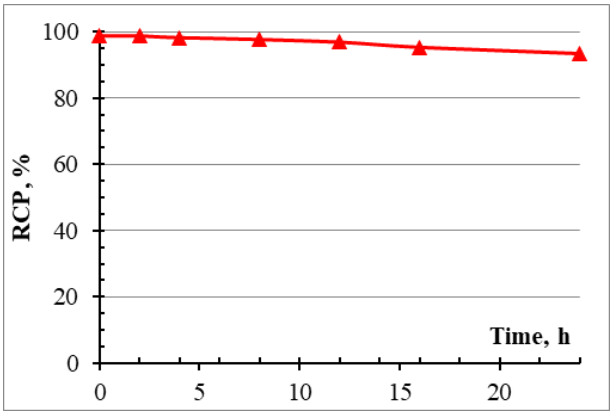

Figure 9. Graf of the study stability of the PhC [PSMA-11].

From the given graph data, it follows that the RCP of the radiopharmaceutical [^{99m}Tc]-PSMA-11 synthesized from the stored lyophilizates of PhC [PSMA-11] for individual doses up to 6 months was more than 98%, and not significant from the values of radiochemical purity on the day of production PhC [PSMA-11]. Hence it follows that the developed method for the synthesis of the PhC [PSMA-11] in the form of a lyophilisate for the preparation of radiopharmaceuticals [^{99m}Tc]-PSMA-11 in individual doses with a storage condition of 3-5 ^{0}C can be set to a expiration date of 6 months.

CONCLUSION

A procedure has been developed for the synthesis of lyophilizate of PhC [PSMA-11] with optimization of the amount of ingredients and sodium pertechnetate ($Na^{99m}TcO_4$) from the ^{99m}Tc Generator to obtain radiopharmaceutical [^{99m}Tc]-PSMA-11 with the highest possible RCY labeling and the RCP of more than 98.5 ± 0.5%.

The conditions for the synthesis of the lyophilizate PhC of [PSMA-11] were studied with the determination of the optimal ratios of ingredients in order to achieve a high RCY of labeling the peptide-ligand of PSMA-11 with the ^{99m}Tc radionuclide. The results of the studies showed that the synthesis of the lyophilizate PhC [PSMA-11] containing the ingredients of the peptide-ligand of PSMA-11, sodium tartrate and L-cysteine was 25 μg; 20 mg and 1.2 mg/vial, respectively.

The stability of radiopharmaceutical [^{99m}Tc]-PSMA-11 in 0.9% NaCl solution and in human serum was determined, that the stability of radiopharmaceutical [^{99m}Tc]-PSMA-11 for more than 4 hours was within 98%, and this is quite sufficient for diagnostic tests studies as a diagnostic tool for PSMA-positive SPECT-CT in men with prostate cancer.

The radiochemical purity of the synthesized radiopharmaceutical [^{99m}Tc]-PSMA-11 from lyophilized of the PhC [PSMA-11] stored at a temperature of 3-5 ^{0}C was determined, and a preliminary expiration storage of the PhC [PSMA-11] of 6 months was established.

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