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Synthesis of pharmaceutical composition of [PSMA-11] and radiopharmaceutical [^{99m}Tc]-PSMA-11 labeled with ^{99m}Tc

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ABSTRACT: This work is devoted to the study of the synthesis of a lyophilizate of a pharmaceutical composition of the [PSMA-11] based on the peptide-ligand of PSMA-11 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 and radiochemical purity. The amount of the peptide-ligand PSMA-11 varied from 10 to 50 µg, ascorbic acid from 10 to 200 mg, and the amount of $SnCl_2$ from 20 to 60 µg, respectively.

The results of the studies showed that the synthesized radiopharmaceutical [^{99m}Tc]-PSMA-11 from the lyophilizate of peptide-ligand of PSMA-11 is the optimal pharmaceutical composition of [PSMA-11] with a peptide-ligand PSMA-11 content of 25 µg and the amount of the added reducing agent $SnCl_2$ 40 µg. The amount of $Na^{99m}TcO_4$ added per pharmaceutical composition was 1,85 GBq. As a result, a radiopharmaceutical [^{99m}Tc]-PSMA-11 with a radiochemical yield and a radiochemical purity of more than 98,5±0,5% was obtained.

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.

KEYWORDS: Technetium-99m, peptide ligand, prostate-specific membrane antigen (PSMA-11), prostate cancer, pharmaceutical composition, radiopharmaceutical of [^{99m}Tc]-PSMA-11, Generator of ^{99m}Tc, half-life, Tin layer chromatography, radiochemical yield, radiochemical purity.

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 study of the synthesis of a pharmaceutical composition with PSMA inhibitor by optimizing the amount of PSMA-11 inhibitor, reducing agent, sodium pertechnetate ($Na^{99m}TcO_4$) from the ^{99m}Tc

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Generator and the pH of the reaction mixture to obtain radiopharmaceutical [^{99m}Tc]-PSMA-11 with the highest possible radiochemical yield (RY) and radiochemical purity (RCP).
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

A. 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. When determining the radiochemical yield (RCY) of the labeling process, a mixture of solvents acetonitrile and chemically pure ethyl alcohol was used as the mobile phase.

B. 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 50-10^4 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 pharmaceutical compositionPSMA-11was 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. Radiochemical purity (RP) and radiochemical labeling yield (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 DISCUSSIONS

A. Synthesis of batches of lyophilizate - kit of PSMA-11

To synthesize the reagent solution of the pharmaceutical composition of [PSMA-11], an aqueous solution 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 radiopharmaceutical, the [PSMA-11] pharmaceutical composition 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. As a result, a radiopharmaceutical [99mTc]-PSMA-11 was obtained with a radiochemical yield and a radiochemical purity of more than 98,5±0.5% n=6.

B. Determination of the radiochemical yield of the process of labeling the pharmaceutical composition - [PSMA-11] with a solution of $Na^{99m}TcO_4$ from the «Generator Tc-99m».

The determination of the radiochemical yield of the labeling process of the pharmaceutical composition [PSMA-11] was carried out by determining the RCP of the radiopharmaceutical [^{99m}Tc]-PSMA-11 by TLC method.

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A plate with a thin layer of silica gel (TLC) was cut into strips 10x100 mm in size, an aliquot of the test solution with a volume of 1,0-1,5 µl with an activity of $7{,}4\times10^5$ Bq was applied to the start line (15 mm from the edge of the strip) and dried on air. The plates were then transferred to a TLC chamber and chromatographed upstream for 20 minutes until the mobile phase reached the finish line using a mixture of acetonitrile with ethanol (CH_3CN:C_2H_5OH) in a phase ratio of 9:1 as the mobile phase. The resulting chromatogram was dried at room temperature, pasted on both sides with adhesive polyethylene tape, cut into 1 cm pieces, and the count rate was measured from the area containing the complex (^{99m}Tc-PSMA-11) and from the entire chromatogram (the sum of the chromatogram pulse counts scc) by the radiometric method. The results of studies on the determination of the radiochemical impurity (RCI) of the radiopharmaceutical [^{99m}Tc]-PSMA-11 are shown in Figures -1 and 2. In the indicated chromatography mode, the retention factor (Rf) of the complex (^{99m}Tc-PSMA-11) and colloids ^{99m}Tc is (0,2 ± 0,1), and Rf of pertechnetate ions, ($^{99m}TcO_4^-$) - 0,9-1,0. The percentage of impurity of pertechnetate ions was determined by counting on the strips developed in a mixture of solvents acetonitrile with ethanol in a phase ratio of 9: 1 as follows:

$$^{99m}TcO_4^- = \frac{^{99m}{}_{TcO_4^-}}{\sum(scc+{}^{99m}{}_{TcO_4^-}+[{}^{99m}Tc]-PSMA-11)} \times 100\% \qquad (1)$$

The determination of the proportion of ^{99m}Tc colloids in the radiopharmaceutical was also carried out by TLC, only as a mobile phase, a mixture of solvents of 1.0 M ammonium acetate solution and methanol was used in a phase ratio of 1:1 by volume.

The procedure was carried out three times for each measurement. The retention factor (Rf) of the ^{99m}Tc colloids was Rf=0,2 and the retention factor of [^{99m}Tc]-PSMA-11 was Rf=0,8.

The percentage of hydrolyzed-reduced ^{99m}Tc (HR-^{99m}Tc) was determined by counting on strips developed in 1M CH_3COONH_4 : CH_3OH with a phase ratio by volume of 1:1

$$HR^{99m}Tc = \frac{HR^{99m}{}_{Tc}}{\sum scc} \times 100\% \qquad (2)$$

$$RCP = 100\% - (^{99m}TcO_4^- + HR^{99m}TcO_4^-) \qquad (3)$$

When determining the radiochemical impurity of the radiopharmaceutical [^{99m}Tc]-PSMA-11 to determine the promotion of $^{99m}TcO_4^-$ ions on the TLP with different developers, in parallel with the radiotracer [^{99m}Tc]-PSMA-11, individual TLPs were applied from a solution of sodium pertechnetate and identically chromatographed.

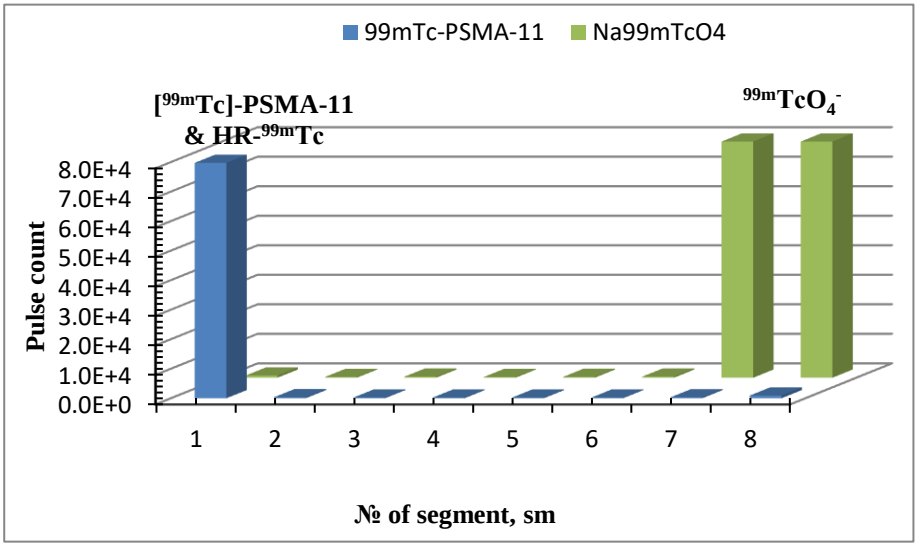

Fig. 1 Distribution of the radiopharmaceutical [^{99m}Tc]-PSMA-11 on TLP «Kieselgel 60», Mobile phase - CH_3CN:C_2H_5OH, n=3;

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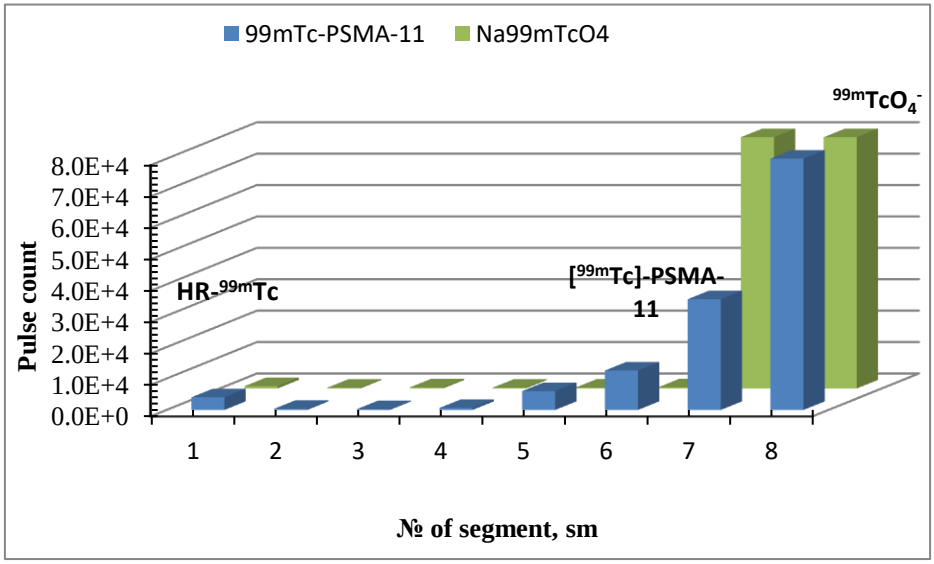

Fig. 1 Distribution of the radiopharmaceutical [^{99m}Tc]-PSMA-11 on TLP «Kieselgel 60», Mobile Phase 1M CH_3COONH_4:CH_3OH, n=3

The obtained results of measurements of the radiochemical purity of the synthesized 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 PSMA-11 pharmaceutical composition and the radioactive pharmaceutical preparation [^{99m}Tc]-PSMA-11 with various amounts inhibitor of the PSMA-11 and ascorbic acid (ASA) 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.

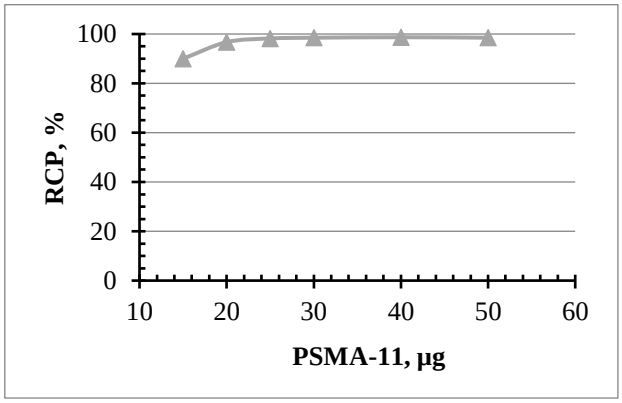

Fig. 3 The influence of the amount of PSMA-11 on the radiochemical labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6

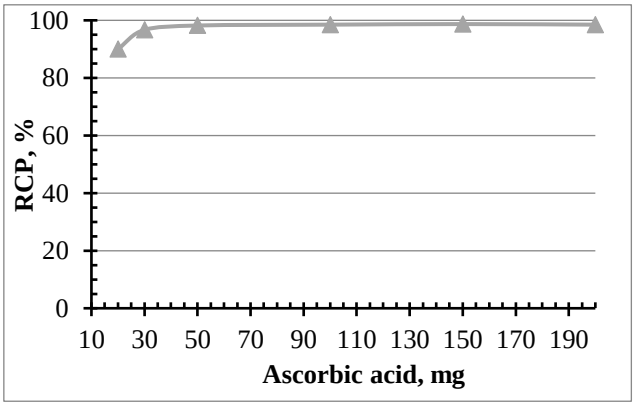

Fig. 4 The influence of the amount of Ascorbic acid on the radiochemical labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6

As can be seen from Figures 3 and 4, when synthesizing a pharmaceutical composition with a PSMA-11 inhibitor content of less than 25 µg, the radiochemical yield of [^{99m}Tc]-PSMA-11 labeling is low, and at more than 25 µg it remains stable, maximum, and in the case of ascorbic acid, a high percentage of radiochemical labeling is observed when the amount of ascorbic acid is 50 mg or more in the pharmaceutical composition. When labeling the pharmaceutical composition of PSMA-11 with technetium-99m with less than 20 µg of the PSMA-11 inhibitor, it promoted the formation of hydrolyzed ^{99m}Tc, and this led to a decrease in RCY labeling. Also, during the labeling process with the amount of ascorbic acid below 50 mg / ml in the reaction mixture, the RHY of the labeling reaction was below 90%, and this is possibly due to

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insufficient capacity of the buffered system. To obtain radiopharmaceutical [^{99m}Tc]-PSMA-11 with a consistently high radiochemical purity, we studied the effect of sodium tartrate and L-cysteine on the yield of radiochemical labeling. To do this, a pharmaceutical composition of [PSMA-11] was synthesized with various amounts of sodium tartrate and L-cysteine ingredients, followed by the synthesis of a radiopharmaceutical [^{99m}Tc]-PSMA-11. The research results are shown in Figures 5 and 6.

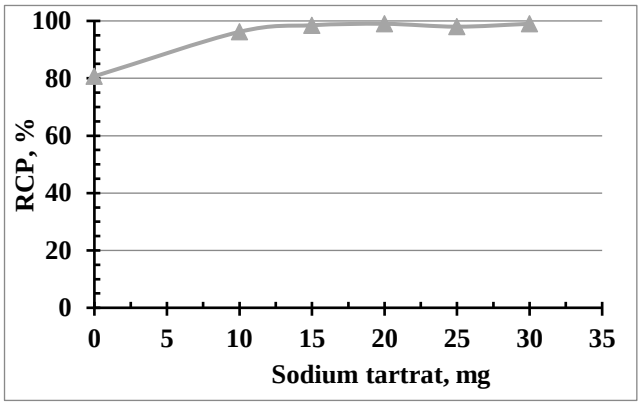

Fig. 5 Influence of the amount of $C_4H_4Na_2O_6$ on the radiochemical labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6

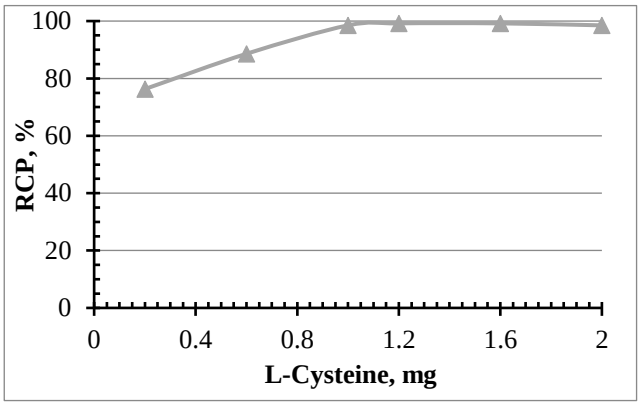

Fig. 6 Influence of the amount of L-Cysteine on the radiochemical labeling of radiopharmaceuticals [^{99m}Tc]-PSMA-11, n=6

It can be seen from the research results (Fig. 5) that during the synthesis of the pharmaceutical composition of [PSMA-11], not including sodium tartrate ($C_4H_4Na_2O_6x2H_2O$) in the kit, the radiochemical yield of [^{99m}Tc]-PSMA-11 labeling is at the level of 70,2 ± 1, 9%, after inclusion of sodium tartrate ingredient ($C_4H_4Na_2O_6x2H_2O$) in the amount of 10 mg/vial into the composition of the pharmaceutical composition of [PSMA-11], the value of the radiochemical yield of labeling [^{99m}Tc]-PSMA-11 increased by 10%, and with an increase in the content of the ingredient wine- acid sodium tartrate to 20 mg/vial, the radiochemical yield of [^{99m}Tc]-PSMA-11 labeling increased to 99,0%.
Also, the inclusion of one more ingredient L-cysteine ($C_3H_7NO_2S$) in the amount of 1,2 mg/vial into the kit during the synthesis of the parmaceutical compositionof [PSMA-11] led to a stable high radiochemical yield of [^{99m}Tc]-PSMA-11 labeling. Thi
s is due to the fact that, apparently, the presence of L-cysteine in the composition of the pharmaceutical composition of [PSMA-11] in combination with sodium tartrate prevents the oxidation of divalent tin during the synthesis by forming a complex with divalent tin.

IV. CONCLUSION

The research conditions for the synthesis of the pharmaceutical composition of [PSMA-11] were studied with the determination of the optimal ratios of ingredients in order to achieve high radiochemical labeling of the PSMA-11 inhibitor with the ^{99m}Tc radionuclide. The results of the research showed that the synthesis of the pharmaceutical composition [PSMA-11] containing ingredients PSMA-11, sodium tartrate and L-cysteine was 25 μg; 20 mg and 1.2 mg/vial, respectively.
Thus, on the basis of the proposed method for the synthesis of the pharmaceutical composition of [PSMA-11], in the aggregate of the proposed ratios of ingredients, the purposed was achieved. As a result, the radiopharmaceutical [^{99m}Tc]-PSMA-11 was obtained with a radiochemical yield and a radiochemical purity of more than 98,0±0.5%.
Based on the research results, a technology for the synthesis of the pharmaceutical composition of [PSMA-11] and the [^{99m}Tc]-PSMA-11 radiopharmaceutical by labeling technetium-99m with a consistently high radiochemical yield and radiochemical purity was developed, and preclinical studies were carried out, but this is the subject of another study.

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REFERENCES

[1]. Cuccurullo V., Stasio G. D. Di, and Mansi L., World Journal Nuclear Medecine, 2018, 17, 70–78.

[2]. Fendler WP, Eiber M, Beheshti M, Bomanji J, Ceci F, Cho S, et al. ^{68}Ga-PSMA PET/CT: Joint EANM and SNMMI procedure guideline for prostate cancer imaging: version 1.0. Eur J Nucl Med Mol Imaging. 2017;44:1014–24.

[3]. Schmidkonz C, Hollweg C, Beck M, Reinfelder J, Goetz TI, Sanders JC, et al. ^{99m}Tc-MIP-1404-SPECT/CT for the detection of PSMA-positive lesions in 225 patients with biochemical recurrence of prostate cancer. The Prostate. 2018; 78:54–63.

[4]. Reinfelder J, Kuwert T, Beck M, Sanders JC, Ritt P, Schmidkonz C, et al. First experience with SPECT/CT using a ^{99m}Tc-labeled inhibitor for prostatespecific membrane antigen in patients with biochemical recurrence of prostate cancer. Clin Nucl Med. 2017;42:26–33.