CHROMATOGRAPHIC METHOD OF SEPARATION OF RADIONUCLIDE LUTETIUM-177, CARRIER FREE FROM MACRO-QUANTITIES OF YTTERBIUM

[1]Akhmedov Zh. A, [2]Usarov Z. O, [3]Abdukayumov A. M, [4]Rikhsiev A. Z, [5]Sherov O. O
State Enterprise "Radiopreparat" INP AS RUz, Tashkent

ABSTRACT

Currently, promising direction of development of modern oncology is a radionuclide therapy using radiopharmaceuticals sighting direction, ie the targeted effect on tumor tissue. For this purpose, suitable radionuclide ^{177}Lu, possessing optimal nuclear-physical characteristics with an average half-life ($T_{1/2}$ = 6,7 days); acceptable energy β-particles (maximum 0,5 MeV), which allows to destroy small tumors and metastasis without affecting the healthy tissues; concomitant soft γ-radiation with sufficient energy for imaging. Targeted exposure helps to get rid of neuroendocrine tumors, thyroid cancers, some types of brain cancer, and prostate cancer, and so on.

KEYWORDS: lutetium, ytterbium, carrier free, half-life, isotop, separation, radionuclide, Chromatographic method, irradiating, thermal neutron, nuclear reactor, nuclear reaction, ion-exchange resin, chromatographic column, HPLC, gradient.

I. INTRODUCTION

One of the reports at the final meeting of the authoritative Society for Nuclear Medicine and Molecular Imaging (SNMMI) [1] in 2019 was completely devoted to the use of targeted therapy with Lutetium-177-PSMA (PSMA-prostate-specific membrane antigen) in prostate cancer. [2]

Radiopharmaceuticals labeled with 177Lu radionuclide of high specific activity are used in radioimmunotherapy and are new generation drugs, which are, in most cases, labeled antibodies or peptides [3].

In this case, the labeling reaction, as a rule, is realized by means of bifunctional chelating agents (BPCA), which can bind to molecules of a biologically active compound on the one hand and, on the other hand, have chelating groups capable of binding metal cations.

The concentrations of biologically active compounds in the composition of the radiopharmaceuticals are extremely small and amount to several micrograms. Therefore, to obtain a high yield in the labeling reaction, the initial solutions of radionuclides, in the ideal case, should not contain impurities of other elements and stable isotopes of the target radionuclide, i.e. a target radionuclide with a high specific activity is needed, namely in the case of ^{177}Lu more than 50 Ci / mg Lu on the day of receipt by the consumer.

Preparation of ^{177}Lu in a nuclear reactor

Radionuclide 177Lu can be obtained in two ways:

- Irradiation of starting material enriched in Lu-176 by nuclear reaction with neutrons from a nuclear reactor

$$^{176}Lu(n,\gamma)^{177}Lu;$$

- Irradiation by neutrons of a nuclear reactor starting material containing Yb enriched in ^{176}Yb by nuclear reaction

$$^{176}Yb(n,\gamma)^{177}Yb \xrightarrow{\beta^-} ^{177}Lu;$$

One of the main disadvantages of the first method is the value of the specific activity of 177Lu, which is significantly lower than the theoretical one, which is proportional to the thermal neutron flux density. In addition, the formation of the "harmful" long-lived radionuclide ^{177m}Lu (T1 / 2 = 160 days) is inevitable, the fraction (by activity) of which in ^{177}Lu can reach 3%. For the effective implementation of this scheme, a highly enriched ^{176}Lu and high-flow reactor (with a thermal neutron flux density of ~ 2-5 × 10^{14} n / cm^2 s^{-1}) is required.[4]

When the second scheme is implemented for a neutron flux of any intensity, ^{177}Lu is obtained with a specific activity close to the theoretical specific activity. Thus, the production of ^{177}Lu using this scheme can be organized on the basis of any research nuclear reactor. To implement this scheme, enriched ytterbium and an express method for separating ^{177}Lu carrier free from the ytterbium target material are required.

Taking into account the advantages and disadvantages of both methods of obtaining ^{177}Lu, the more preferable in our case is the method of obtaining ^{177}Lu by irradiating ytterbium targets. In this case, the target ^{177}Lu is obtained carrier free, which can be purified and concentrated using radiochemical methods.

This work presents the results of experiments on the separation of carrier free amounts of ^{177}Lu from the target material of ytterbium by ion-exchange and extraction-chromatographic methods in a dynamic mode.

II. EXPERIMENTAL

The irradiated material was used as the ytterbium nitrate with a natural isotopic composition and ytterbium oxide enriched ytterbium-176 (99,8%) The samples were irradiated in vertical channels of the WWR-SM research reactor (INP AN RUz). The irradiation times of the samples were 48 and 120 effective hours of ytterbium nitrate and ytterbium oxide, respectively. The irradiated sample was kept for 24 hours to decay the activity of the short-lived radionuclide ^{177}Yb ($T_{1/2}$ = 1,911 hours).

Reagents: All reagents used in this work were of the highest purity (unless otherwise stated).

E-ISSN NO:2349-0721

Radiochemical procedures for the isolation, purification, and concentration of 177Lu were performed by extraction and ion exchange chromatography. As the extractant, in the experiments, it was used di-2-etilgeksilortofosfornaya acid (D2EHPA).

Commercial D2EHPA was preliminarily purified from the mono fraction by the method described in [5]. As the carrier liquid extractant, in the case of an extraction chromatographic experiments was used a powder of tetrafluoroethylene FT(4) with particle size 400 mesh.

The solid extractant D2EHPK / FT (4) for extraction chromatography was prepared according to the procedure [5].

The ion-exchange resin was a DOWEX 50x8, 200-400 mesh, (Sigma Aldrich) cation-exchange resin in the H^+ form, which was converted into the NH_4^+ form by treatment with a 0,5 M NH_4CI solution. As the complexing agent was used alpha-hydroxyisobutyric acid (α-HIBA) (Sigma Aldrich).

The extraction chromatographic column had dimensions d = 6 mm and H = 500 mm, and in the case of ion exchange chromatography the dimensions of the columns were d = 6 mm and H = 750 mm; and d = 10 mm and H = 500 mm.

The process of ion exchange chromatography isocratic and gradient mode carried out by liquid chromatography on Gilson HPLC pumps 305. Measurement of medium of the solution was carried out on a Seven Easy pH meter. Measurements of the quantitative and qualitative activities of radionuclides were carried out on an ASPECT SU-03P gamma spectrometric device with a semiconductor Ge (Li) detector. Radionuclides were identified by their gamma lines.

III. RESULTS AND DISCUSSION

Figure 1 illustrates the extraction chromatographic separation of carier free 177Lu radionuclide from the Yb target material.

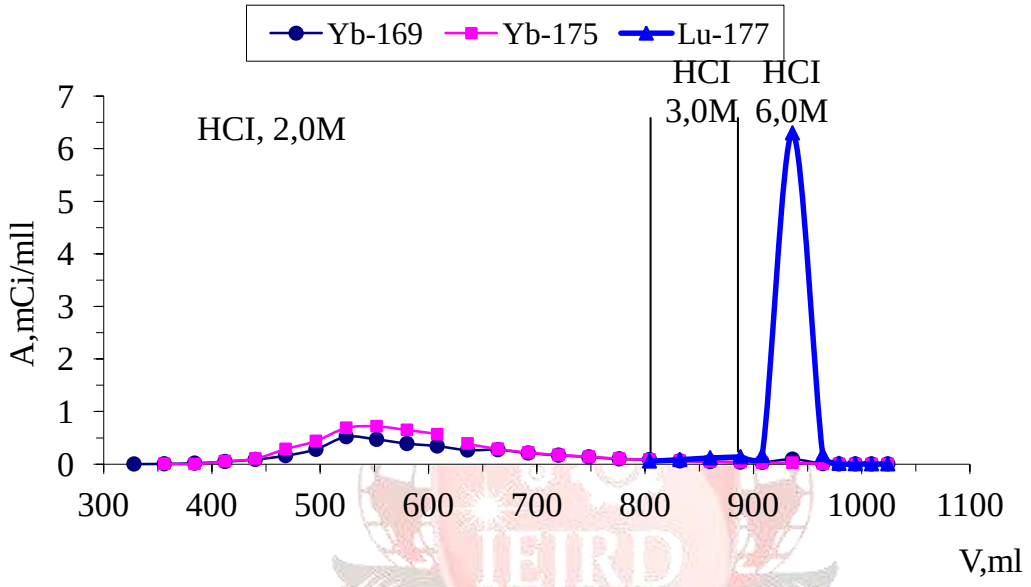

Fig. 1. Profile elution of ^{169,175}Yb and ^{177}Lu from a chromatographic column (D2EHPK/FT-4). C_{Yb} = 10 mg. Elution rate 1,0 ml/min.

As can be seen from the figure, the elution of ytterbium from a chromatographic column with a solid extractant of fluoroplastic (FT-4) impregnated on it with an extractant of di-2-ethylhexylphosphoric acid (Di-2-EHPA) was carried out by elution with 2,0 M HCI solution, at a rate of 1,0 ml / min with a volume of 800 ml. Elution of residual amounts of ytterbium with 3,0 M, HCl solution, volume 100 ml, and elution of lutetium-177 was carried out with 6,0 M HCl solution, volume 100 ml. In this case, the percentage of separation of non-relative amounts of ^{177}Lu from the ytterbium target material was more than 98%.

In order to obtain the ^{177}Lu radionuclide, without a carrier with a high specific activity free of ytterbium impurities, for the manufacture of radiopharmaceuticals of protein origin, at least three-fold separation by the above method is required. And this inevitably leads to a decrease in the radiochemical yield of the final product.

In this regard, we conducted a study on the separation of lutetium-177, carrier free from macro-quantities of the target material ytterbium by ion exchange chromatography.

Figures 2-5 depict the ion exchange separation of the radionuclide of ^{177}Lu carrier free from the irradiated sample of ytterbium target material. For this purpose, DOWEX 50x8 200-400 mesh cation exchange resin, in the H^+ form, was transferred to the NH_4^+ form by treatment with a 0,5 M NH_4CI solution with a pH of -3,5-5,0.

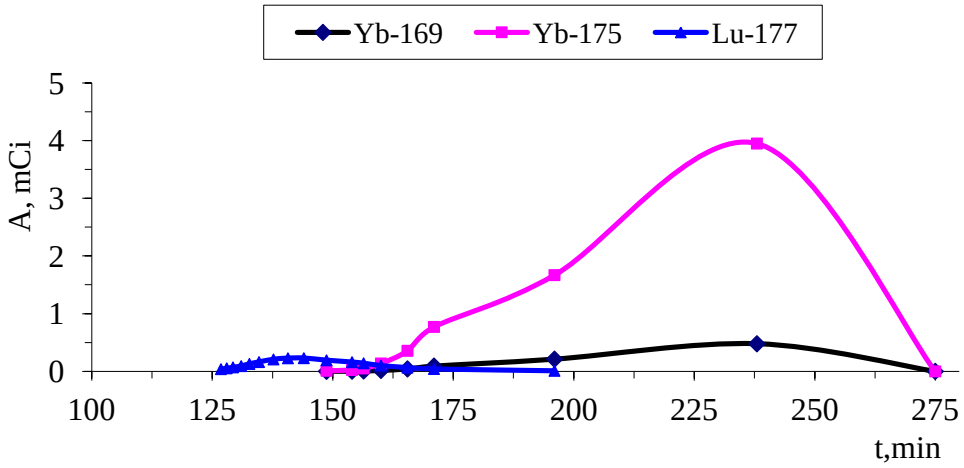

Fig. 2. Elution profile of chromatographic separation of ^{177}Lu from 6,0 mg of natural Yb target material using DOWEX 50x8 200-400 mesh, NH_4^+ form chromatographic column. (column size: ID = 6mm, h = 750mm).

Figure-2. Illustrates desorption of ^{177}Lu and ytterbium ions. Desorption was performed with a complexone α- hydroxyisobutyric acid in the range of pH =3,5-5,0 in isocratic mode. The amount of the loaded irradiated sample of ytterbium, natural composition and the concentration of the eluent of α-hydroxyisobutyric acid was C_{Yb} = 6,0 mg, and $C_{\alpha HIBA}$ = 0,08 mol/L, respectively, with the activity of radionuclides Av_{177Lu} = 1,64mCi; Av_{169Yb} = 10,02 mCi; Av_{175Yb} = 136,1mCi the elution rate is 0,2 ml/min.

In this case, the sequential desorption of $^{177}Lu^{3+}$ ions, and then $^{169,175}Yb^{3+}$, occurred, as a rule, according to the values of the stability of the complex compounds ^{177}Lu:(α-HIBA)$_3$ > ^{169,175}Yb:(α-HIBA)$_3$ with the α-HIBA complexone, which increases with increasing atomic number in the Yb-Lu series, respectively.

Under these conditions of separation of ^{177}Lu from macro-quantities of ytterbium, was obtained 1,22 mCi of ^{177}Lu carrier free, which was 74% of the initial activity of lutetium-177.

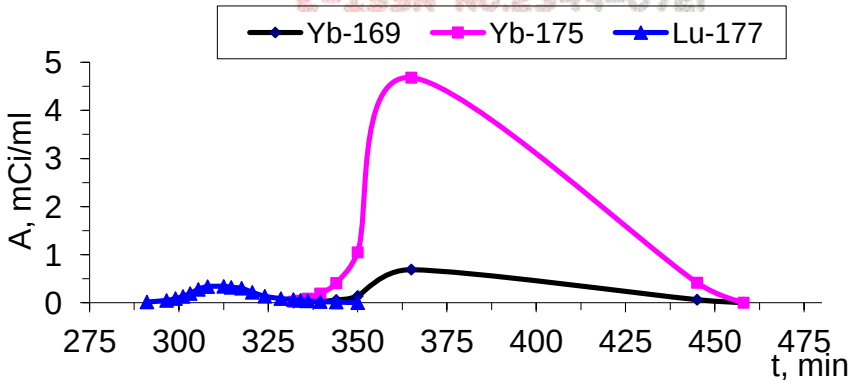

Fig. 3. Elution profile of chromatographic separation of ^{177}Lu from 20 mg of natural Yb target solution using DOWEX 50x8 200-400 mesh, NH_4^+ form chromatographic column.

Figure-3. depicts the desorption of 177Lu and ytterbium ions from a chromatographic column with dimensions Ø = 10mm, h = 500mm with a strongly acidic cation exchanger DOWEX 50x8 200-400 mesh, in NH4 + form, be the complexone α-HIBA in a gradient mode. The amount of loaded irradiated sample of ytterbium of natural composition was 20,0 mg. The activities of the loaded initial radionuclides were Av_{177Lu} =

3,38 mCi; Av_{169Yb} = 3,37 mCi; Av_{175Yb} = 35,3 mCi respectively. The mode of changing the concentration of the eluent during the elution of radionuclides is shown in table-1.

Under these conditions of separation of ^{177}Lu, without a carrier, 3,17 mCi of ^{177}Lu was obtained from macro-quantities of ytterbium, carrier free, and this amounted to 94% of the initial activity of lutetium-177.

Under these conditions of separation of ^{177}Lu, carrier free, was obtained 3,17 mCi of ^{177}Lu from macro-quantities of ytterbium, and this amounted to 94% of the initial activity of lutetium-177.

Table-1 Mode of gradient of separation of ^{177}Lu and Yb.

№	Time, min	Flow rate of eluent, ml/min	H_2O, %	0,132 M α-HIBA, %
1	000,0	0,30	62	38
2	360,0	0,80	40	60
3	600,0	0,80	0	100

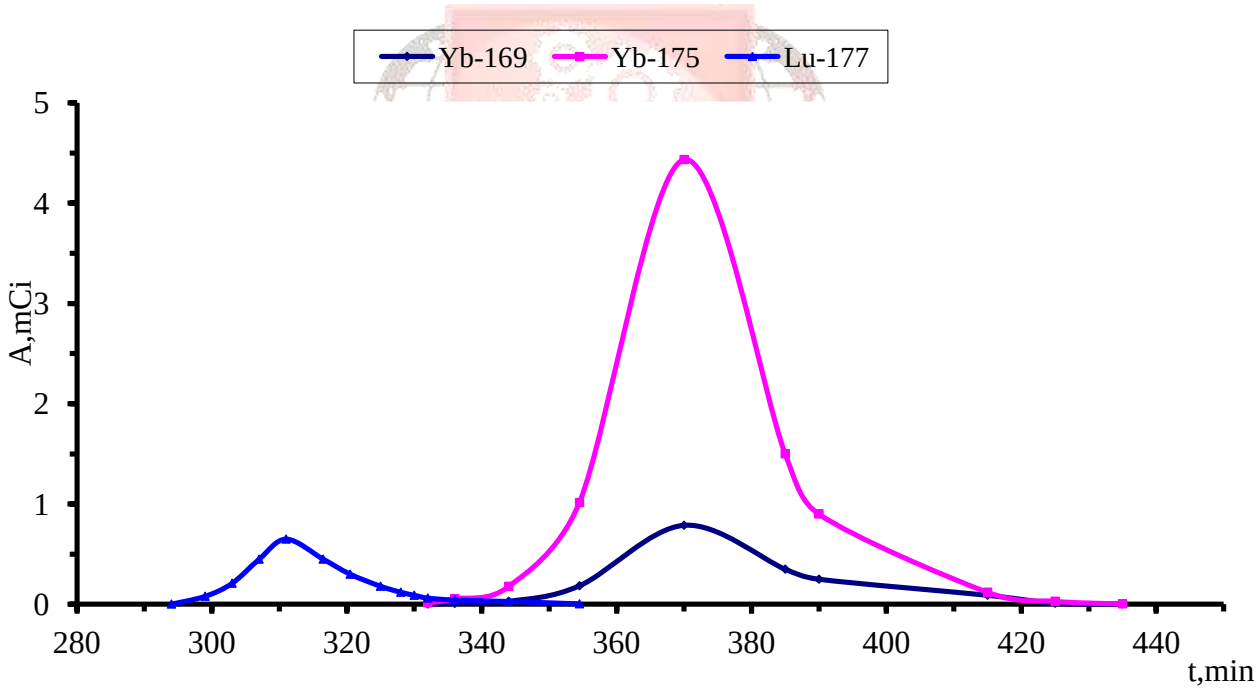

Fig. 4. Elution profile of chromatographic separation of ^{177}Lu from 50 mg of natural Yb target solution, using DOWEX 50x8 200-400 mesh, NH_4^+ form chromatographic column. (column size: ID = 10 mm, h = 500mm).

Figure 4 illustrates the separation of ^{177}Lu and ytterbium from a chromatographic column. Elution was carried out with α -HIBA chelator in a gradient mode. The amount of the loaded irradiated sample of ytterbium of natural composition was 50,0 mg with the activities of the initial radionuclides Av_{177Lu}=3,9 mCi; Av_{169Yb}=2,87 mCi; Av_{175Yb}=30,6 mCi. The eluent gradient mode is shown in Table-2. As can be seen from Figure 4, after elution of lutetium at 350 minutes of the process, the supply of the complexing agent concentration in the chromatograph was increased to 100%, which led to a narrowing of the peak of ytterbium.

Under these separation conditions, 3,5 mCi of ^{177}Lu was obtained carrier free, which was 91% of the initial activity of lutetium-177.

Table-2 Mode of gradient of separation of ^{177}Lu and Yb.

№	time, min	Flow rate of eluent, ml/min	H_2O, %	0,132 M α-HIBA, %
1	000,0	0,30	62	38
2	350	0,60	41,7	58.3
3	600	0,60	0	100

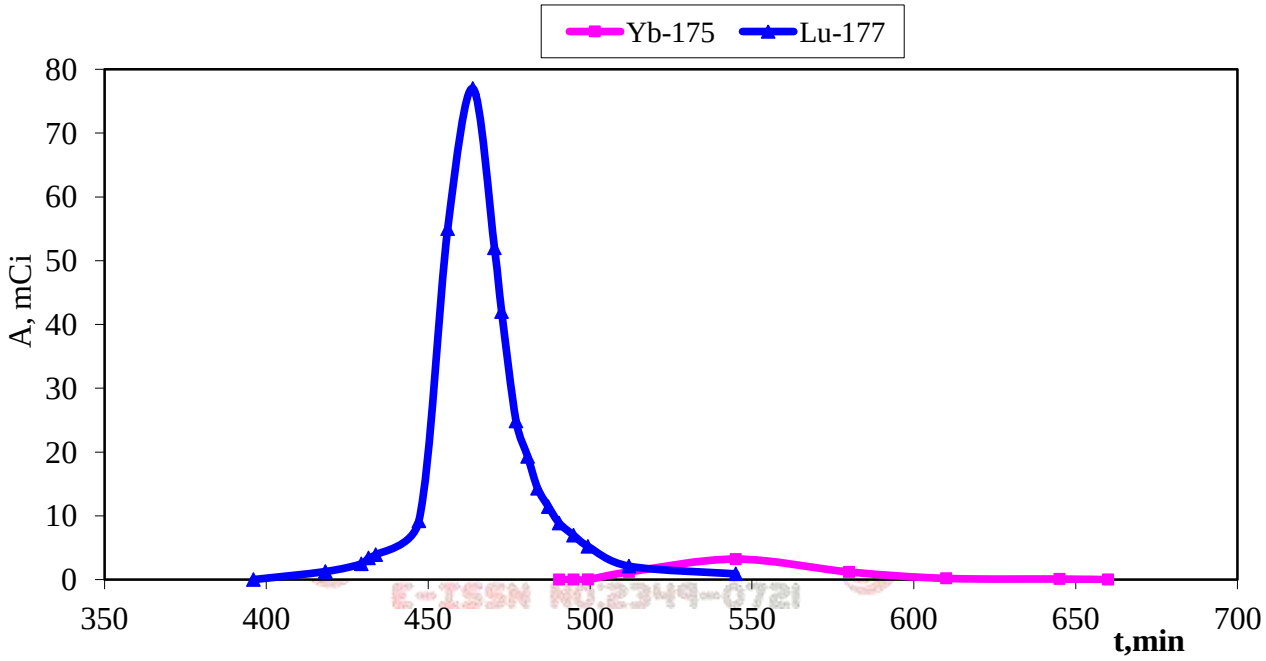

Fig. 5. Elution profile of chromatographic separation of ^{177}Lu from 70 mg of enriched ^{176}Yb target solution, using DOWEX 50x8 200-400 mesh, NH_4^+ form chromatographic column. (column size: ID = 10 mm, h = 500mm).

Figure 5 illustrates the process of separation of ^{177}Lu from an irradiated ytterbium sample enriched in ytterbium-176, on a chromatographic column, with the complexing agent α-HIBA in a gradient mode. The eluent gradient mode is shown in Table-3. The amount of the loaded irradiated sample of ytterbium was 70,0 mg with the activities of the initial radionuclides Av_{177Lu} = 871 mCi; Av^{175Yb} = 36,7 mCi.

Table-3 Mode of gradient of separation of ^{177}Lu and Yb.

№	time, min	Flow rate of eluent, ml/min	H_2O, %	0,132 M α-HIBA, %
1	000,0	0,40	62	38
2	600	0,40	0	100

3	500,0	1,0	0	100

With these separation conditions, 784 mCi of ^{177}Lu carrier free was obtained, which was 90% of the initial activity of lutetium-177.

Further, to purify the resulting lutetium-177 from chelator, ammonium ions and other accompanying metal ions, the fractions with lutetium-177 were acidified to pH-2 with concentrated HCI solutions and passed through a chromatographic column with DOWEX 50x8 200-400 mesh, in H^+ form with column size Ø = 5mm, h =10 mm.

Then the column was washed with three column volumes with deionized water, 2,0 mol/l HCI solution and eluted with 4,0 M HCI solution, then the eluate with lutetium-177 was evaporated on a rotary evaporator to dryness and dissolved in the required amount of 0,04 M HCI solution. The measurement results of the finished ^{177}Lu product are shown in Table 4.

Quality certificate for the preparation Lutetium chloride ($^{177}LuCI_3$), carrier free in 0,04 M HCI solution.

Table 4.

№	Analysis name	Specification	Results of the analysis
1	Appearance	Colorless, clear solution	Colorless, clear solution
2	Radioactive concentration, mCi/ml	≥1000	2000
3	Specific activity, Ci/mg	≥ 50	92,6
4	Radionuclide purity, all gamma emitting impurities, (^{58}Co, ^{60}Co, ^{65}Zn, ^{54}Mn, ^{59}Fe, ^{51}Cr), %	^{175}Yb and all impurities ≤ 0,01	0
6	Radiochemical purity, %	≥ 99,0	99,6
7	Hydrochloric acid concentration, mol/l	0,04	0,04
7	pH	1-2	1,37

CONCLUSION

In this work, we investigated the conditions for the separation of non carrier added of the radionuclide lutetium-177 from macro-quantities of the target material ytterbium by extraction and ion-exchange chromatographic methods.

The most optimal conditions for the separation of the radionuclide lutetium-177, carrier free, turned out to be the method of ion-exchange chromatographic separation with a complexone α-hydroxyisobutanoic acid at pH =3,5-5,0 in a gradient. When separating lutetium-177 from large gram amounts of ytterbium, the main part of ytterbium can be discharged by electrolysis on a mercury cathode, but this is already the subject of research in another work.

Thus, from the obtained results of the work, it can be concluded that the method of separation of the radionuclide lutetium-177, with a high specific activity, is suitable for the synthesis of radiopharmaceuticals based on proteins.

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