AL-FARABI KAZAKH NATIONAL UNIVERSITY

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Development of a radiopharmaceutical based on Lu-177 labelled elagolix for therapy and diagnosis

6D060600 – «Chemistry»

Dissertation submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy (Ph.D.)

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The Republic of Kazakhstan
Almaty, 2020

CONTENT

NORMATIVE REFERENCES

In this dissertation, references to the following standards are used:

GOST 61-75. Reagents. Acetic acid.

GOST 11125-84. High purity nitric acid.

GOST 199-78. Reagents Sodium acetate 3-aqueous.

GOST 4328-77. Reagents. Sodium hydroxide.

GOST 4233-77. Reagents. Sodium chloride.

GOST 3118-77. Reagents. Hydrochloric acid.

GOST 6824-96. Glycerin distilled.

GOST 3117-78. Reagents. Ammonium Acetate.

GOST 3652-69. Reagents. Citric acid monohydrate and anhydrous.

GOST 10652-73. Reagents. Disodium ethylenediamine-N, N, N ', N'-tetraacetic acid salt, 2-aqueous (Trilon B).

GOST 624-70. Salicylic acid (2-hydroxybenzoic), Tech.

GOST 4459-75. Potassium chromate.

GOST 10163-76. Reagents. Soluble starch.

GOST 1277-75. Reagents. Silver nitrate.

GOST 20298-74. Ion-exchange resins. Cationites.

GOST 215-73. Laboratory glass mercury thermometers.

GOST 28365-89. Reagents. The method of paper chromatography.

GOST 19808-86. Glass medical. Stamps.

GOST 1770-74. Measured glass laboratory glassware. Cylinders, beakers, flasks, test tubes.

GOST 25336-82. Measured glass laboratory glassware.

GOST 16327-88. Laboratory ware and equipment made of glass. Types, basic parameters and dimensions.

GOST 29227-91. Laboratory glassware. Graduated pipettes.

GOST 20477-86. Polyethylene tape with an adhesive layer.

GOST R 51268-99. Scissors. General specifications.

GOST 892-89. Tracing paper. General specifications.

GOST 12026-76. Laboratory filter paper.

GOST R 51314-99. Caps aluminum and combined for corking medicines

GOST 6.38-90. Unified documentation systems. The system of organizational and administrative documentation. Requirements for documents.

GOST 7.1-2003. Bibliographic record. Bibliographic description. General requirements and compilation rules.

GOST 8.417-2002. State system for ensuring the uniformity of measurements. Units of physical quantities.

GOST 7.11. System of standards on information, librarianship, and publishing. Abbreviation of words and phrases in foreign European languages in bibliographic

description.

GOST 7.9-95. System of standards on information, librarianship, and publishing. Thesis and abstract. General requirements.

GOST 24104-2001. Laboratory balance. General technical requirements.

TC 48-4-417-87. Scandium oxide stamps.

TC38-6108-95. Rubber products.

CRM 7837-2000. Zinc in 1M nitric acid.

CRM 7875-2000. Manganese (II) in 1M nitric acid.

CRM 7834-2000. Chromium (VI) in 1M nitric acid.

CRM 7873-2000. Nickel in 1M nitric acid.

CRM 7836-2000. Copper in 1M nitric acid.

CRM 7877-2000. Lead in 1M nitric acid.

CRM 7835-2000. Iron (III) in 1M nitric acid.

CRM 7927-2001. Aluminum in 1M nitric acid.

CRM 7143-95. Arsenic (III) in 0.2 M sulfuric acid.

CRM 7238-96. Tin (IV) in 0.1 M hydrochloric acid.

SP RK. State Pharmacopoeia of the Republic of Kazakhstan.

Rules of labeling of medicines. Order of the Minister of Health and Social Development of the Republic of Kazakhstan dated 04.16.2015 No. 227 (as amended on 04/22/2019).

Rules of Good Manufacturing Practice of the Eurasian Economic Union.

Sanitary and epidemiological requirements for radiation safety, approved by order of the Minister of Health of the Republic of Kazakhstan dated June 26, 2019 No. ҚРДСМ-97.

Sanitary and epidemiological requirements for radiation hazardous facilities, approved by order of acting Minister of National Economy of the Republic of Kazakhstan No. 260 of 03/27/2015.

Rules for the transport of radioactive materials and radioactive waste", order of the Minister of Energy of the Republic of Kazakhstan dated 02.22.2016, No. 75.

DESIGNATIONS AND ABBREVIATIONS

BC	– breast cancer
TNBC	– triple-negative breast cancer
HER2 (+/-)	– human receptors for epidermal growth factor (expression, lack)
ER	– estrogen receptor
PR	– progesterone receptor
HER2/neu	– gene located on the long arm of chromosome 17 (17q12 – q21)
GnRH	– gonadotropin releasing hormone
GnRHR	– gonadotropin releasing hormone receptor
PC	– paper chromatography
HPLC	– high performance liquid chromatography
RCP	– radiochemical purity
RSCI	– Russian Science Citation Index
ELAGOLIX (ELA)	– gonadotropin releasing hormone antagonist, 4 - [[(1R) -2- [5- (2-fluoro-3-methoxyphenyl) -3 - [[2-fluoro-6- (trifluoromethyl) phenyl] methyl] -4- methyl-2,6-dioxopyrimidin -1-yl] -1-phenylethyl] amino] butanoic acid
DOTA	– tetraxetan
^{177}Lu –DOTAELA	– ^{177}Lu - 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid - 4 - [[(1R) -2- [5- (2-fluoro-3-methoxyphenyl) -3 - [[2-fluoro-6-(trifluoromethyl) phenyl] methyl] -4-methyl-2,6-dioxoprimidin-1-yl] -1-phenylethyl] amino] butanoicacid
FDA	– Food and Drug Administration - Agency of the Ministry of Health and Social Services
USA	– United States of America
RK	– The Republic of Kazakhstan
HCC1806, HCC1937	– positive for the epithelial cell specific marker Epithelial Glycoprotein 2 (EGP2) and for cytokeratin 19. The cells are negative for expression of estrogen receptor (ER) and for expression of progesterone receptor (PR)
AEZS-108	– zoptarelin doxorubicin(developmental code names AEZS-108,AN-152) consists of doxorubicin linked to a small peptide agonist to the luteinizing hormone-releasing hormone(LHRH) receptor
AEZS - 125	– (disorazol Z-D-Lys6-LHRH conjugate) is a novel cytotoxic hybrid based on the natural compound Disorazol Z and the LHRH receptor
MDA-MB-231	– is a highly aggressive, invasive and poorly differentiated triple-negative breast cancer (TNBC) cell line as it lacks estrogen

	receptor (ER) and progesterone receptor (PR) expression, as well as HER2.
MDA-MB-453	– is an androgen-responsive human breast carcinoma cell line with high level androgen receptor expression
SKBR3	– is a human breast cancer cell line that over expresses the Her2
MCF-7	– is a human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors
EFO-27	– human ovarian adenocarcinoma cell line established from the solid omental metastasis of a mucinous papillary adenocarcinoma
OV-1063	– is an epithelial ovarian cancer cell line
In vitro	– (latin for within the glass) refers to the technique of performing a given procedure in a controlled environment outside of a living organism
In vivo	– (latin for "within the living") refers to experimentation using a whole, living organism as opposed to a partial or dead organism
FSH	– follicle-stimulating hormone
mRNA	–matrixribonucleic acid
PCR	– Polymerase chain reaction
LH	– luteinizing hormone
hCG	– human chorionic gonadotropin
RP	– radiopharmaceutical
DNA	– Deoxyribonucleic acid
REE	– rare earth elements
BFC	– bifunctional chelator
FHMA	– ferric hydroxide macroaggregates
DOTATOC	– is a promising clinical PET radiotracer for detection of neuroendocrine tumors that express somatostatin receptors
MIBG	– methiodobenzyl guanidine
HA	– hydroxyapatite
PET	– positron emission tomography
SPECT	– single photon emission computed tomography
LET	– linear energy transfer
RBE	– relative biological effectiveness
DTPA	– diethylenetriaminepentaacetic acid
EDTA	– ethylenediaminetetraacetic acid
MeV	– megaelectronvolt
KeV	– kiloelectronvolt
Ci	– Curie
GBq	– gigabecquerel
CRM	– certified reference material
RSGS	– reference spectrometric gamma sources

FN1	– type of chromatographic paper
UV	– ultraviolet
FID	– flame ionization detector
GC	– gas chromatography
WWR-K	– water-water reactor of Kazakhstan
MCNP6	– is a general-purpose, continuous-energy, generalized-geometry, time-dependent, Monte Carlo radiation-transport code designed to track many particle types over broad ranges of energies
ENDF/B-VII.1	– the most recently released set of evaluated nuclear data files produced by the US Cross Section Evaluation Working Group
JEFF-3.2	– The Joint Evaluated Fission and Fusion Nuclear Data Library
FA1, FA2, CR	– different configuration in reactor zone
HEPES	– (4- (2-hydroxyethyl) -1-piperazineethanesulfonic acid) is a zwitterionic organic buffering agent
SPE	– solid phase extraction
EC	– exclusion chromatography
IEC	– ionexchange chromatography
LLE	– liquid-liquid extraction
FDG	– fluorodeoxyglucose
BFCA	– bifunctional chelating agent
DOTATATE	– is a compound containing tyrosine3-octreotate, an SSR agonist, and the bifunctional chelator DOTA
SSR	– somatostatin receptors
SepPac C18	– cartridges based on silica gel with strong hydrophobicity
SCX	– strong cation exchanger used to separate bases
Dowex 50W-X-12	– ion exchange resin in H^+ form (50–100 mesh)
PuroliteWCA 100	– ion exchange resin with a high exchange capacity, in Na^+ - form
КУ-2	– cation exchange resin, multifunctional strongly acidic resin in Na^+ form
RCB	– radiochemical building
AW	– auxiliary work
TP	– technical process
PLS	– packaging, labeling, shipping
QC	– quality control
RCPC	– radiopharmaceutical production casing
SP RK	– State Pharmacopoeia of the Republic of Kazakhstan
LAL	– limulus amebocyte lysate
IU	– international unit
UKTIA	– type of transport packaging

RIPC INP ME RK	– Radiochemistry and Isotope Production Center Institute of Nuclear Physics ME RK
GMP	– good manufacturing practice
MES RK	– Ministry of Education and Science of the Republic of Kazakhstan
SERRS	– sanitary and epidemiological requirements for radiation safety
AG	– analytical grade
HPGe	– high purity germanium
NA	– neutron activation

INTRODUCTION

General characteristics of research work.

The thesis «Development of a radiopharmaceutical based on Lu-177 labelled elagolix for therapy and diagnosis» is devoted to the development of a procedure forproducing a radiopharmaceutical based on antagonist of gonadotropin-releasing hormone for the diagnosis and treatment of triple-negative breast cancer.

The relevance of the study.

Breast cancer (BC) is a malignant neoplasm of the glandular tissue of the breast. Despite the decrease in mortality due to the introduction of preventive examinations and effective systemic hormonal chemotherapy, about 1 million new cases are registered annually in the world.

In women in many countries of the world, including Kazakhstan, breast cancer (BC) ranks 1st among other types of malignant neoplasms. On average, about 4 000 breast cancer patients are diagnosed annually in the Republic of Kazakhstan, and more than 1 380 women among them die. In particular, 4 142 new cases of breast cancer were registered in 2014, which amounted to 22.4 per 100 000 of the population. The lethality for 1 year is 8.2%, and the 5-year survival rate is 55.8%. The specific weight of BC I-II stages in 2013 was 77.1%, and BC IV stage was 4.9% [1].

Triple negative breast cancer (TNBC) accounts for 8-20% of breast tumors. A special feature is that triple negative breast cancer occurs in women under the age of fifty years before menopause and even during the first pregnancy in the early period, and then after childbirth, it may be accompanied by short-term breastfeeding. Also occurs early in menarche and with high body mass index. Cells of this type are characterized by a lack of expression of receptors for estrogen, progesterone and HER-2, which makes it difficult to choose a therapeutic vector that is aggressive, the maximum risk of recurrence within three years after surgery, metastasis, and a decrease in life expectancy.

The thrice negative phenotype includes a breast tumor subtype that is clinically negative for expression of estrogen and progesterone (ER and PR) receptors and is negative for the human epidermal factor (HER2) receptor protein, with unique prognostic and therapeutic indices. Unlike other subtypes, the target agents specifically targeted for thrice-negative breast cancer are not yet available, which increases the need and interest in advancing new therapeutic strategies beyond the chemotherapy for this subgroup of high-risk patients[2-4].

Modern world treatment protocols provide for surgical intervention in order to remove the primary tumor and part of the lymph nodes into which malignant cells are likely to have spread. A combination of several treatments is also performed, such as hormone therapy, chemotherapy and radiation therapy. After surgery to remove the removal of breast cancer, there are no less important stages, such as high-precision radiation therapy with chemotherapy. Unfortunately, side effects limit the effectiveness

of chemo-radio-therapy. The use of selective drugs makes it possible for effective treatment, which includes the determination of biochemical processes, as a result of which there is a differentiation of tumor tissue from healthy tissue [5-8].

The thrice negative breast cancer expresses the receptors for gonadotropin-releasing hormone (GnRH) in more than 50% of cases [2]. Among several analogues (agonists and antagonists) of GnRH, that have been studied for therapy, the most interesting is the non-peptid antagonist elagolix. Elagolix is the first of a new class GnRH inhibitors that were designated as the second generation due to their non-peptidic nature and peroral bioavailability. Due to the relatively short half-life of elagolix, the action of gonadotropin-releasing hormone (GnRH) is not completely blocked during a day period. For this reason, the levels of gonadotropin and sex hormones are only partially suppressed, and the degree of suppression can be dose-dependent adjusted when required. Moreover, if administration of elagolix is stopped, its effects are quickly reversible. Moreover, the frequency and severity of menopausal side effects, such as hot flashes, also decrease compared to the first-generation GnRH inhibitors [9-13].

The purposes of the thesis: radiolabeling of DOTAELA with ^{177}Lu with the following development a potential radiopharmaceutical for diagnosis and treatment of triple-negative hormone-sensitive breast cancer.

The tasks of the thesis:

- Production of lutetium-177 with high and proper specific activity by neutron activation for further using in radiolabeling.
- Radiolabeling DOTAELA with produced lutetium-177 and determination the optimal synthesis of the DATOELA-^{177}Lu complex condition with high radiochemical purity.
- Selection and verification of quality control methods and preparation of draft specifications for the production of pilot batches.

The object of the thesis: radiometallic chelate for the diagnosis and treatment of triple-negative hormone-sensitive breast cancer based on the elagolix - antagonist of gonadotropin-releasing hormone.

The subject of the research: the interaction of radioisotopes with chelating agents, determining the technological parameters of the synthesis at the product yield, quality control of the drug being developed.

The scientific novelty of the thesis

1. A paper chromatography system was selected to evaluate the yield of ^{177}Lu-DOTAELA. In the course of the work, such factors as chromatography paper, mobile phase, pH, composition and ratio of the components of the mobile phase were studied.

This system was further used in assessing the radiochemical purity of the ^{177}Lu-DOTAELA complex.

2. Optimal technological parameters of ^{177}Lu-DOTAELA synthesis were determined.

3. The composition of the reaction mixture and the block scheme for producing a radiopharmaceutical based on DOTAELA have been developed. The finished product has a radiochemical purity of greater than 95%, which proves its suitability for medical and biological tests.

4. Methods of analytical quality control of the radiopharmaceutical “177Lu-DOTAELA” have been developed. Methods for the qualitative and quantitative determination of the main components in the composition were adopted and tested. A draft specification for a new radiopharmaceutical has been created.

The methodological framework of the research

Investigations were carried out with the help of generally accepted scientific and experimental methods. The experiments to determine the effect of temperature, time and composition on the technological output of the synthesis reaction, as well as testing of the optimal method for purifying of the synthesized batches, was performed in a sealed "hot" cell. To determine the technological output at the synthesis stage, the gamma spectrometry methods (gamma spectrometer EGPC 30-185-R), thin-layer chromatography was used to determine the radiochemical purity, the effect of the solvents composition and their residual quantity in the product, the method of gas chromatography (Agilent 7890A gas chromatograph GC), as well as high-performance liquid chromatography was used for determination of chemical purity (Agilent 1260). Apyrogenity was determined with the help of the LAL-test, and the sterility was determined by direct seeding on nutrient media, followed by incubation of the samples for 14 days at 25 °C and 37 °C.

The scientific and practical significance of the study

The radiopharmaceutical material will find application in nuclear medicine for the diagnosis and treatment of TNBC. The results of this work will stimulate the development of highly informative and minimally invasive methods of nuclear medicine and contribute to the creation of new domestic RP for the diagnosis and treatment of various diseases.

The theoretical significance of the results

In the process of completing the thesis, a procedure was developed for the preparation of the dosage form of the radiopharmaceutical «^{177}Lu-DOTAELA», methods for monitoring its quality of the main components, and a draft Specification was proposed for the production of pilot batches of the radiopharmaceutical to conduct its subsequent preclinical studies at the University of Oslo.

The validity and reliability of the results

All results are reliable and reasonable since all measurements were carried out on calibrated instruments using standard methods using gamma spectrometry, atomic emission spectrometry, and paper chromatography. All conclusions are compared with the facts of the experiment.

Part of the study was carried out at the University of Oslo (Norway) under the supervision of Professor Patrick Riss.

The validity and reliability of the results are provided by metrological processing of the results of experimental data.

The main provisions submitted to the defense of the thesis

1. Obtaining the source of ^{177}Lu with the desired activity and purity, for elagolix labelling, produced by the neutron activation reaction through ^{176}Lu (n, γ)^{177}Lu at the WWR-K reactor with specific activity 819 GBq / mg Lu-177 with a thermal neutron flux of $1.2 \cdot 10^{14}$ $cm^{-2} \cdot s^{-1}$.
2. Optimal parameters for the synthesis^{177}Lu radiolabelled DOTAELA at pH-4.5 with radiochemical yield 50.1 %, temperature 90-100° C with radiochemical yield 95.3%, complexation time 40 minutes with radiochemical yield 50.1%.
3. A block diagram of the ^{177}Lu-DOTAELA complex preparation, showing target preparation, synthesis conditions and packing leading to RCP ≥ 95% and a draft specification for the development of its pilot batches.

Approbation of work

The results of the thesis were reported and discussed at international symposia and scientific conferences such as:

- 11th International Conference «Nuclear and Radiation Physics», September 12-15, 2017 in Almaty, Kazakhstan;
- International scientific conference of students and young scientists «Farabi Әlemi», Almaty, Kazakhstan, April 9-12, 2018;
- 19th reporting scientific and technical conference of the Institute of Nuclear Physics, December 18-19, 2018 in Almaty, Kazakhstan;
- III international scientific-practical conference «Actual problems of the development, production and use of radiopharmaceuticals - RADIOFARMA-2019», Moscow, June 18 - 21, 2019;
- I international conference «Nuclear and Radiation Technologies in Medicine, Industry, and Agriculture» June 24-27, 2019 in Almaty, Kazakhstan;
- The 20th reporting scientific and technical conference of the Institute of Nuclear Physics, December 18-19, 2019 in Almaty, Kazakhstan.

Publications

Based on the results of the thesis 10 scientific papers were published, including:

Gurin A.N., Patrick J Riss, Chakrova E.T., Uralbekov B.M. Development of quality control of the DO3A-NBI-56418 labelled by ^{177}Lu for theranostic goals of triple negative cancer with using paper chromatography // 11th International Conference "Nuclear and Radiation Physics", Almaty, 2017

Gurin A.N., Soloninkina S.G., Riss P., Uralbekov B.M., Matveyeva I.V., Chakrova E.T. Selection of mobile phase systems for chromatographic research of ^{177}Lu-DOTAELA. // Chemical journal of Kazakhstan. - 2018.- Vol. 2. - P. 151-157.

Gurin A.N., Soloninkina S.G.3rd International Scientific Conference of Students and Young Scientists «Farabi Alemi», Almaty, Kazakhstan, April 9-12, 2018. «On the issue of the influence of pH synthesis on the radiochemical purity of the labelled compound DOTAELA -^{177}Lu», 6 section, p. 245.

Gurin A.N., Chakrova E.T., Riss P. Purification of DOTAELA labelled with^{177}Lu // Proceedings of the III international scientific-practical conference «Actual problems of the development, production and use of radiopharmaceuticals - RADIOFARMA-2019» / Ed. G.E. Kodina and A.Ya. Maruk - M.: FSBI SSC FMBC them. A.I. Burnazyan FMBA of Russia, 2019.- P. 61.

Gurin A.N., Chakrova E.T., Riss P. Study of the stability of the^{177}Lu-DOTAELA // Abstracts of the 1st international conference «Nuclear and Radiation Technologies in Medicine, Agriculture Industry» - Almaty. - 2019. P. 219.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. Quality control test for ^{177}Lu-DOTAELA. International Journal of Biology and Chemistry, [S.l.], v. 12, n. 2, p. 112-115, 2019. ISSN 2409-370X.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. et al. Study of the purification of ^{177}Lu-DOTAELA complex // Pharmaceutical Chemistry Journal. 2020. Vol. 54. No. 1, P. 64-68 (Q4, IF – 0.51).

Gurin A.N., Riss P., ChakrovaYe.T., Matveyeva I. V. Development of a ^{177}Lu radionuclide-labelled bioconjugate, a gonadotropin releasing hormone antagonist // Medical physics. 2020. Vol. 85. No. 2, P. 17-23.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. Optimization of Reaction Parameters for the Synthesis of ^{177}Lu DOTAELA // Revista de Chimie. 2020.Vol. 71. No. 8, P. 55-62.

Gurin A.N., Chakrova Ye.T., Matveyeva I. V. Patent №5186 for utility model «Method for radiochemical labelling of an organic compound with the lutetium-177 isotope», application No2020/0213.2 dated 27.02.2020 of RSE «National institute of intellectual property» (Republic of Kazakhstan).

The personal contribution of the author

Direct participation in research and the general formulation of tasks, in the analysis and statistical processing of the results; writing articles and reports, testing and introducing research results into the development of a new domestic radiopharmaceutical.

Relation of the thesis with research and government programs

This dissertation work was performed and supported within the framework of the grant funding project of MES of Republic of Kazakhstan AP 05134384«Determination of the optimal technological parameters for preparation of a new radiopharmaceutical for diagnosis and therapy of thrice-negative breast cancer (TNBC) with an elagolix-^{177}Lu of antagonistic mechanism of action» (2018-2020 yy.).

Volume and structure of the thesis

The thesis consists of an introduction, three sections, a conclusion, and a list of references. The work is presented on 100 pages (with appendix 103), contains 38 figures, 16 tables, and 137 bibliographical references.

1 LITERATURE REVIEW

1.1 Triple-negative breast cancer

In the work of T. Sørlie et al. [14], the main subtypes of breast cancer associated with changes in the expression profile of certain genes involved in the development of breast cancer are identified. The study was performed on 78 patient-derived breast cancer samples and the expression of 1753 genes was investigated, allowing for statistical analysis using a clustered hierarchical method. In total, four distinct groups of patients were identified with some significance ($p<0.001$): luminal subtype A, luminal subtype B, basal-like subtype (TNBC) and HER-2 positive subtype, each of which has a different prognosis and different approaches for therapy [15]. The following table provides an overview of biomarkers used for molecular classification [16], which allowed for characterizing the subtypes:

Table 1 – Molecular subtypes of breast cancer and key changes in the genotype of the tumor.

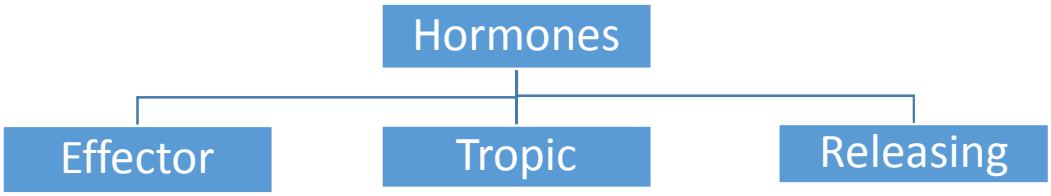
Molecular subtype	Expression of receptors
Luminal A	ER^+и/или PR^+, $HER\text{-}2^-$
Luminal B	ER^+и/или PR^+, $HER\text{-}2^+$
$HER\text{-}2^+$	ER^-, PR^-, $HER\text{-}2^+$
Basal-like	ER^-, PR^-, $HER\text{-}2^-$

The selection of subtypes of breast cancer makes sense not only for the choice of therapy and evaluation of the effectiveness of treatment, but also for prediction, including long-term survival rates, without recurrence [14].

In particular, breast cancer is divided into hormone-sensitive and hormone insensitive forms. For the hormone-sensitive breast cancer, the presence of estrogen and progesterone receptors in the tumor tissue is characteristic. Such tumors often proceed relatively well: a decrease in relapse rate compared to more pronounced delayed benefit is observed in the first five years of continued tamoxifen therapy [15].

In contrast to endocrine- dependent breast cancer, hormone- insensitive tumors are characterized by the absence or low expression of steroid hormone receptors. This type of breast cancer is characterized by rapid growth, aggressive course, as well as lack of effect of anti-estrogen treatment [16, 17].

Hormones can be classified by functionality as shown in the following figure 1.



Affect the target organ	Regulation of synthesis and release of effect or hormones. Stand out adenohypophysis	Regulate the synthesis and secretion of adenohypophysis hormones, mainly tropic. Stand out by the nerve cells of the hypothalamus

Figure 1 – Hormone classification according to role and function

TBNC is insensitive to hormones the frequency of which is 10-24 %, due to the aggressiveness of the clinical course of this cancer and a small number of molecular targets it is one of the most studied subtypes in recent years. Molecular-genetic analysis of TNBC phenotypes showed that only 70% of these tumors are basal-like, the remaining 30% are other biological subtypes [18, 19]. It should be noted that the concepts of "triple-negative" and "basal-like" are not identical. Most breast cancers with basal cell differentiation have a triple negative phenotype. However, 5% of them express estrogen receptors, 6-12% express HER2 / neu [20].

In women under 50, TNBC is found before menopause, at the beginning of menarche, the first pregnancy at an earlier age, a short period of breastfeeding, and a high body mass index [21-24]. A feature of TNBC is the lack of expression of the receptor for estrogen, progesterone and HER-2, which complicates the management of the disease, and as a result, there is an aggressive course, a high probability of relapse during the first years after surgery, metastasis and a decrease in the duration and quality of life [26,27].

Unlike other subtypes, drugs, which are specifically aimed at a triply negative breast tumor are not yet available, which reinforces the need and interest in promoting new therapeutic strategies beyond the limits of chemotherapy for this subgroup of high-risk patients**.**

Many drugs interact with biomolecules in the body. As a result, there is a change in the functional ability of these biomolecules. As a result, biochemical processes with a pharmacological effect occur. The biomolecules with which drugs interact are called receptors, which in turn are proteins.

For example, receptors for hormones, growth factors, mediators, proteins that are involved in biological processes in the body. Nucleic acids with which anticancer drugs interact can also act as receptors.

The receptors are of pharmacological importance. Target receptors interact with many drugs, usually with high receptor specificity. Agonists are drugs that, when binding

to a receptor, reproduce a physiological effect, while drugs that do not cause such an effect, but interfere with the binding of endogenous ligands, are called antagonists [27].

Gonadotropin-releasing hormone (GnRHR) receptors were first discovered in the pituitary gland [28]. GnRHRs have also been found in various human malignant tumors, such as breast, prostate, ovarian, and endometrial cancers [29, 30]. In benign neoplasms, these receptors were practically not detected or were at a low level. This has served as the basis for much work on the development of targeted therapy for specific treatments. GnRH peptide analogs can be used as targeted therapy by disrupting the autocrine stimulation loop based on locally produced GnRH and its corresponding receptor, thereby slowing down tumor growth [29, 30]. Accordingly, in GnRHR, which expresses tumors of the endometrium, prostate, colorectal cancer, lungs, and ovaries, the proliferation of cancer cells is inhibited directly by antagonistic analogs of GnRH [28, 30]. Many GnRH analogs are now commercially available and have been approved by the FDA for other indications, on this basis, clinical trials can be carried out faster [31]. Another strategy was to create a new class of anticancer drugs in which compounds based on a GnRH analog and a cytotoxic compound, such as doxorubicin, are used for targeted chemotherapy of GnRHR-positive cancer[32-34]**.**In works devoted to TNBC, it is noted that more than 50% of TNBC samples express GnRH receptors [38,39].

In 2009, Dr. Stefan Buchholz et al. published the results of studies that examined the expression of gonadotropin-releasing hormone on HCC1806 and HCC1937 TNBC cell samples. Of 17 samples of TNBC according to immunohistochemical analysis, only 2 had a high concentration of receptors, 3 low and the remaining 11 medium. GnRH antagonist Cetrorelix was used in the studies. When using in vitro 1 μm Cetrorelix after 72 hours, a decrease in proliferation of 20 and 18% HCC1806 and HCC1937, respectively, was observed. An in vivo study after 28 days showed a decrease in tumor growth of up to 53% compared with the control group [33].

Already in 2011, an article by Dr. Carsten Grundker et al., describes 2 experiments. In the first experiment, the object of the study was three groups of mice carriers of triply negative breast cancer HCC1806. One group was the control, the second was injected with 60 nmol / 20 g of doxorubicin and the third was administered 60 nmol / 20 g of the AEZS-108 releasing hormone agonist. On the 15th day of the research, truly unique results were obtained. In the group where the GnRH agonist was used, the tumor was 88.0 ± 9.7% (100%; $P < 0.001$) of the initial size. With the use of doxorubicin, the tumor volume increased to 716.0 ± 43.1% (100%; $P < 0.001$), in contrast to the initial values and the control group, the increase reached 1420 ± 77.6% (100%; $P < 0.001$). The second experiment completely repeated the previous one, except for a variety of grafted cells of the MDA-MB-231 type. The results of the second experiment showed a similar situation. When using the AEZS-108 releasing hormone agonist, the tumor decreased to 72.4 ± 18.7% (100%; $P < 0.001$) in comparison with the initial tumor volume. In the group where doxorubicin was used, the tumor increased to 459.6 ± 45.7% (100%; $P < 0.001$), and in the control group to 890.0 ± 53.4% (100%; $P < 0.001$) [34].

C.W. Kwok et al. 2014 published data on an equally interesting study of the effects of several materials with different concentrations separately and synergies, such as the GnRH agonist triptorelin, disorazol-Z, as well as the GnRH conjugates AEZS-125, perifosine, etc. The study used cell lines of the type MDA-MB-231 and HCC1806. The study confirms the possibility of using GnRH receptors as a target in targeted therapy, and the use of combined materials can lead to a higher therapeutic effect [2].

Already by 2010, a series of studies with the active IG1 monoclonal antibody trastuzumab (trade-name Herceptin) aimed at human epidermal growth factor receptor 2 ($HER2^+$) were carried out, however, MTCV is characterized by $HER2^-$. S. Rasaneh et al. produced Herceptin radiolabeling with lutetium-177 and showed in their work the effect of various concentrations of ^{177}Lu-Herceptin, from 0.03 μg / ml to 4 μg / mL, on cell survival. At a concentration of 4 μg / mL, the relative number of surviving cells decreased to 36 ± 3.5% (on the second day) and very slowly increased to 43 ± 4.9% on the seventh day. At Herceptin concentrations (≥0.5 μg / mL), cell growth was rapid. The relative number of surviving cells after applying ^{177}Lu-Herceptin concentration of 4 μg / mL, the relative number of SKBR3 cells decreased to 14 ± 2% and 5 ± 0.9% on the second and seventh day of post-injection, respectively. The complex showed cytotoxicity about eight times compared with natural Herceptin [35]. In 2019, an article was published by M. Kameswaran et al. in which SKBR3 cells (high expression, $HER2^+$), as well as MDA-MB-453 (low expression, $HER2^+$) and MDA-MB-231 ($HER2^-$), were used as targets. The results showed that with the action of ^{177}Lu-Herceptin on SKBR3 cells, binding of 23.3 ± 1% was observed, which was inhibited to 9.0 ± 0.2% (61.4% inhibition), in MDA-MB-453 cells the binding was observed at 13.1 ± 0.8%, which was inhibited to 4.6 ± 0.6% (64.9% inhibition). MDA-MB-231 cells, which are triple-negative cell lines, did not show any binding or inhibition [36].

1.2 Gonadotropin-releasing hormone (GnRHR), agonists and antagonists

Gonadotropin-releasing hormone (GnRH), figure 2) was discovered in 1971. GnRH agonists and antagonists are used in the treatment of disease such as prostate carcinoma, breast cancer, endometriosis, uterine fibroids, and protocols that support reproductive etechnologies [37]. GnRH is a decapeptide that is synthesized and accumulates in the hypothalamus, from where it is released in a pulsed mode into the portal system.

Combined with the plasma receptors of pituitary gonadotrophs, GnRH stimulates the synthesis and secretion of luteinizing (LH) and follicle-stimulating (FSH) hormones. The mammalian GnRH receptor is a seven-component transmembrane portal-type.

Data on the presence of GnRH receptors in normal human extrahypophyseal tissues, including the mammary glands, placenta, ovaries, and testes, are contradictory. Northern blot analysis did not detect mRNA of the GnRH receptor in any sample of extrahypophyseal tissues. However, when using reverse transcriptase PCR, this type of mRNA is found in granulosa cells [38].

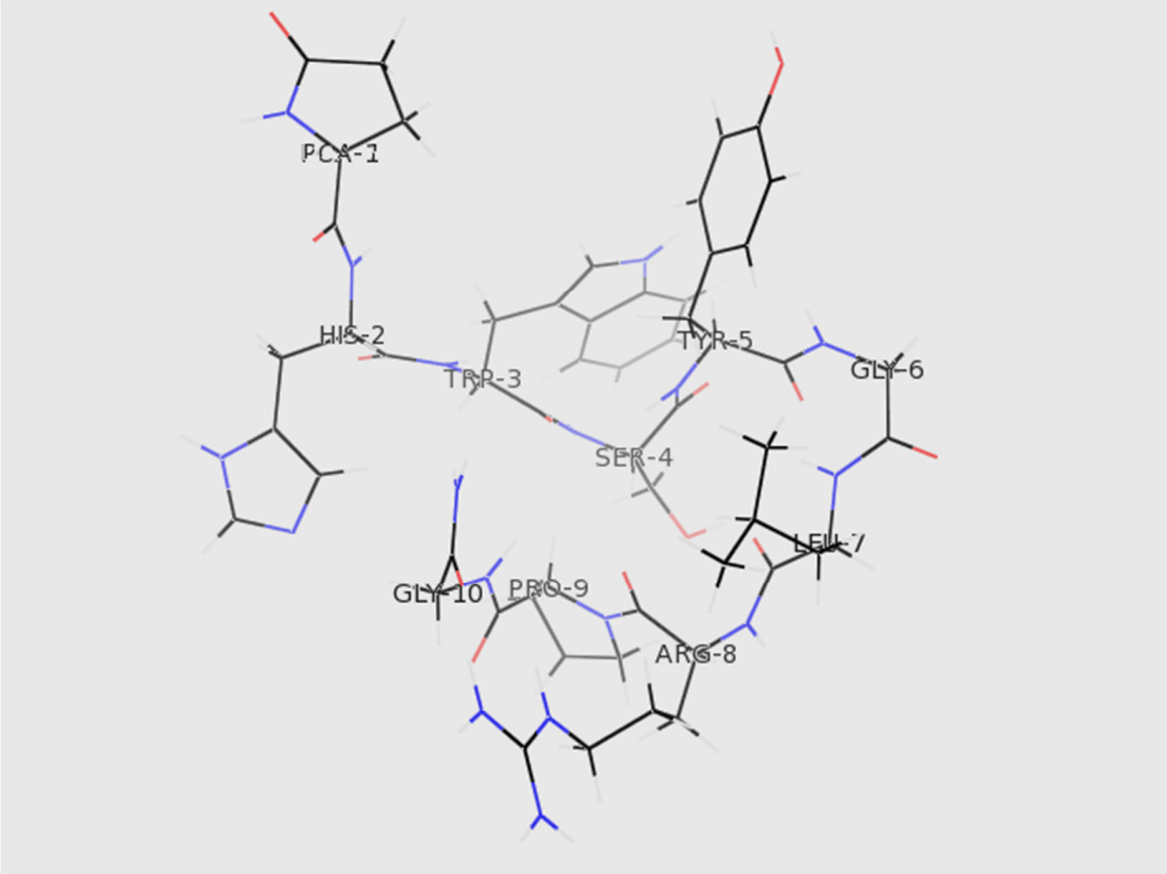


Figure 2 - Gonadotropin-releasing hormone [39]

Overstimulation by GnRH has a detrimental effect on the level of its receptors, while LH and hCG inhibit the expression of GnRH receptors on granulosa cells. Regulation of the GnRH receptor level is tissue-specific, and the role of GnRH as an autocrine regulatory system in the ovary has been proven, in addition to the well-known role of GnRH as a neuroendocrine regulator in the anterior pituitary [40].

Expression of GnRH-receptors occurs in various extrahypophyseal tissues, in particular, in human trophoblast, blood mononuclear cells, ovaries and granulosa cells, testicles, various regions of the brain. Signal transmission from the GnRH receptor in normal extrahypophyseal tissues continues to be studied, but the mechanisms already discovered coincide with those in the pituitary gland [41].

Early studies have shown that breast, ovarian, endometrial, pancreatic and prostate cancer cells express GnRH specific receptors. These receptors differ from the pituitary by reduced affinity and a high degree of seizure. Later it turned out that in cell lines of breast cancer, ovarian cancer, endometrium and prostate gland, as well as in the studied early biopsy samples, there are two types of binding molecules for GnRH: One type with reduced affinity and a high degree of seizure, the second with inverse characteristics. The authors noted the expression of mRNA of the GnRH receptor in the breast cancer cell line

MCF-7 [42]. In samples of ovarian and endometrial cancer cell lines, expression of GnRH receptor mRNA of the high affinity, low ability to capture type was detected. It was found that the nucleotide sequence of the GnRH receptor in the tissues of breast and ovarian tumors is the same as that of the pituitary receptor. The data available today suggests that about fifty percent of breast cancers and about 80 percent of ovarian and endometrial cancers express pituitary receptors for GnRH (high affinity) [43].

The direct inhibitory effect of GnRH agonists on the in vitro proliferation of breast cancer culture cells was first demonstrated by Blankenstein et al. and Miller et al. some researchers have shown the presence of a dose- and time-dependent inhibition of proliferation of various cancer cell lines in vivo by actions of GnRH agonists and antagonists. In most cancer cells, besides the ovarian cancer cell line EFO-27, GnRH antagonists acted as agonists, leading to the arguable conclusion that there are no differences between GnRH agonistic and antagonistic analogues in tumor cells [44]. Using human ovarian cancer (OV-1063) xenografts in mice, Yano et al. showed significant inhibition of tumor growth against the background of a long-term administration of the GnRH antagonist cetrorelix, and the absence of such an effect against the background of the agonist triptorelin. Since both GnRH analogs cause a comparable suppression of the pituitary-ovarian axis, the authors suggested that the antitumor effect of cetrorelix is caused by a direct effect on the receptors in the tumor. Detection of the direct antitumor effect of GnRH analogs in ovarian and endometrial cancer cells has been described by other researchers, in full or partial agreement with the results presented above. Conversely, other researchers could not fix the antitumor effect of GnRH analogues in human ovarian and endometrial cell lines or observed it only at extremely high concentrations of GnRH analogs [45].

Gonadotropin-releasing hormone agonists differ from natural GnRH by replacing amino acids at position 6 (at the site of proteolysis) and often the presence of an ethylamide group instead of a glycinamide group at the C-terminus of the molecule. These modifications lead to large stability improvements against hydrolysis and increase the biological half-life in circulation [46]. According to the mechanism of influence, GnRH agonists include two phases: the phase of short-term stimulation, characterized by an increase in the level of LH, FSH in the blood and, as a result, an increase in estradiol and, and to a small extent, progesterone. As a result, receptor depletion occurs when the blood; and a pituitary desensitization phase. There is a resistance of gonadotrophs to stimulation and the level of gonadotropins in the blood, which decreases. The phase and dose of GnRH antagonists was determined the intensity and duration of the second phase [47].

The mechanism of action of GnRH antagonists is the opposite of agonist action. After introduction GnRH antagonists competitively block the receptors of GnRH in the pituitary gland. In contrast to GnRH agonists, antagonists act immediately (a decrease in the level of LH is observed within a few hours) and bind strongly to the GnRH receptor without causing its activation [48].

Based on the foregoing, it can be concluded that the presence of GnRH receptors in breast cancer cells, increases the likelihood of the accumulation of GnRH analogs labelled with radioactive indicators, which in turn creates prospects for the development of radioactive agents for diagnosis and therapy of tumors. Thus an incentive for research on the topic is created.

Figures 3, 4, 5 show several examples from a wide variety of commercially available GnRH analogues. Among GnRH analogues, nonpeptide antagonists are of particular interest. Of particular interest is due to the fact that these representatives have a lower molecular weight compared to GnRH agonists and peptide antagonists, which in turn affects the clearance of a compound in the body.

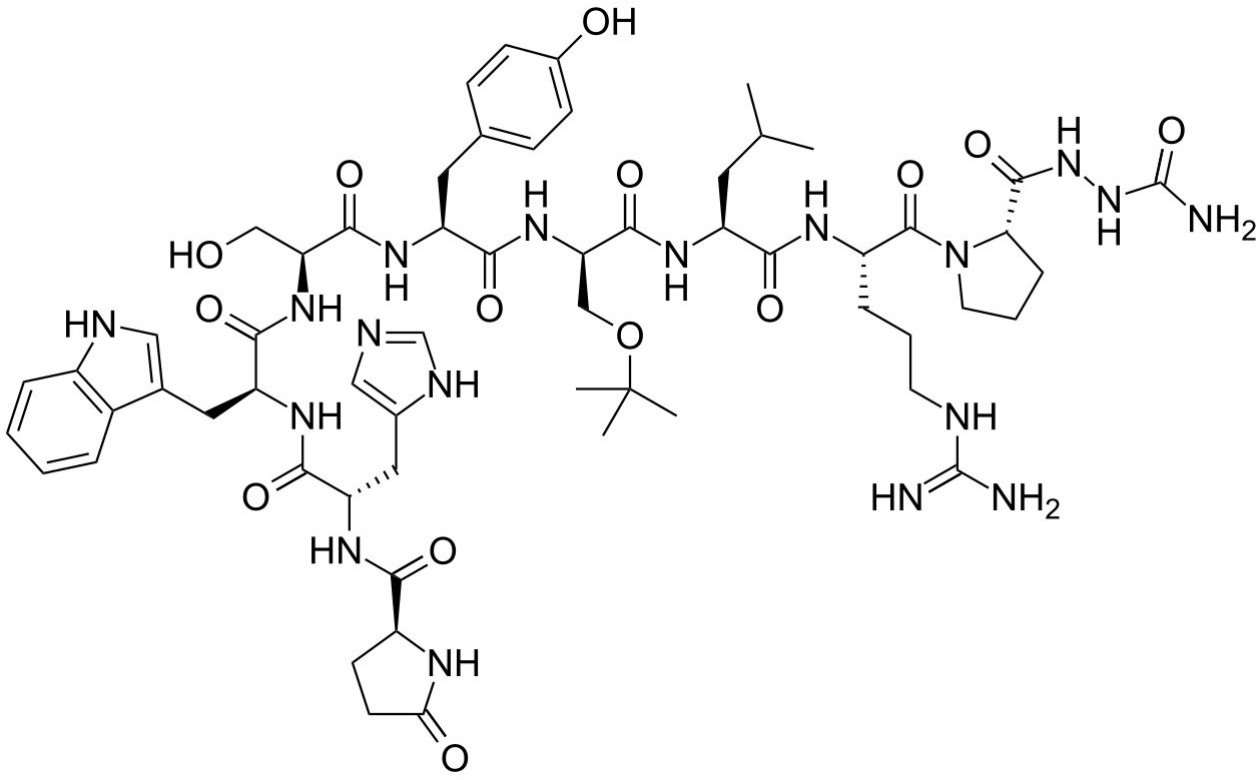


Figure 3 – GnRH agonist goserelin

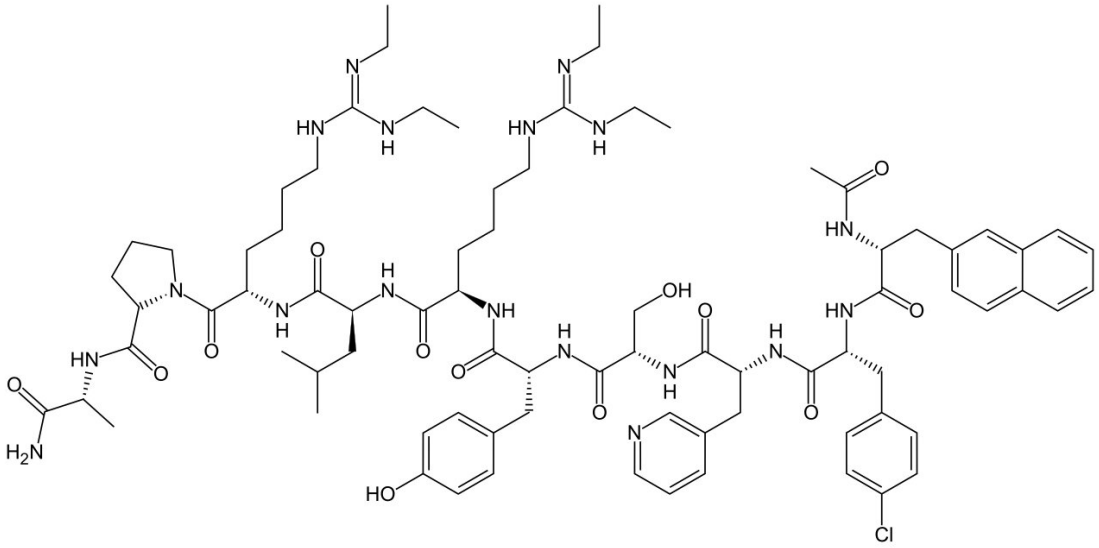
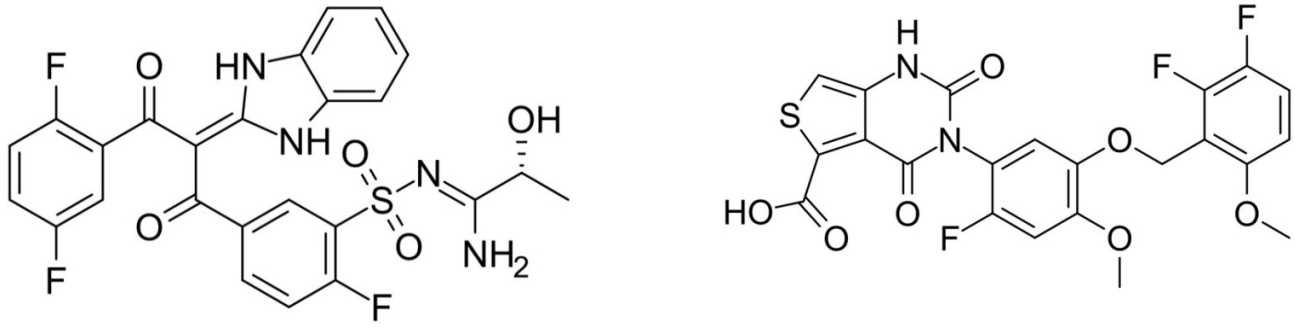
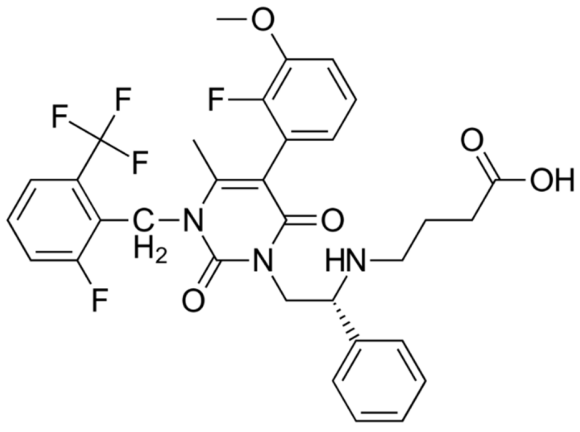


Figure 4 - GnRH peptide antagonist ganirelix



A B



C

Figure 5 - Non-peptidic GnRH antagonists: A - Opigolix; B - Linzagoliks; C - Elagolix

1.3 Targeted radionuclide therapy

Targeted radionuclide therapy is based on the specific accumulation of a pharmaceutical drug containing a radioactive isotope only in the tissues of the tumor focus. The main goal of targeted radionuclide therapy is to directly affect tumor cells. This criterion makes it possible to develop a pharmaceutical drug with a large, and, ideally, an infinitely large therapeutic index. This opens up great opportunities for high efficacy with few side effects. In general, the proposed method of treatment is an ideal case; in practice, many difficulties are encountered. This is due to the fact that there is little damage to normal tissues, which occurs in the process of "approaching the target" due to the "bystander effect" and the catabolism of the pharmaceutical drug, accompanied by the release of the radioisotope [49].

After the injection of a pharmaceutical preparation (within a few minutes), it acts on the tumor for several days; the patient does not undergo additional medical procedure sat this time [50]. The development of a radiopharmaceutical product with a prolonged action and a high therapeutic index requires the selection of a radioisotope and a carrier molecule for its delivery to the tumor.

The first mention of the therapeutic use of ^{131}I was back in 1941 [51]. The drug has shown efficacy in the treatment of hyperthyroidism and differentiated thyroid cancer, as well as differential specific absorption of selective radiation (mainly from β-particle emissions) to the thyroid gland or thyroid tumor.

The radioactive anion (^{131}I-) is a vivid example, which shows that widespread tumors can be systematically directed to a radiopharmaceutical with sufficient specificity to completely eradicate the metastatic disease in humans [52].

1.4 Radiopharmaceuticals and requirements

Among the radiopharmaceuticals used for the diagnosis and targeted therapy of tumors, there are:

1. Radiopharmaceuticals that can accumulate in the cells surrounding the neoplasm:
- in intact tissues;
- tissues changed under the influence of malignancy.
2. Radiopharmaceuticals that can accumulate on neoplasm cell membranes:
- by the mechanism of "antigen-antibody";
- the mechanism of interaction with cell receptors.
3. Radiopharmaceuticals that penetrate neoplasm cells:
- specific;
- non-specific.
4. Lymphotropic radiopharmaceuticals for visualization of sentinel lymph nodes [53].

The suitability of the radiopharmaceutical is determined by the biological characteristic of the reflection of the functions of the organism or an individual organ.

However, this criterion is not paramount, since at present it has become possible to include radionuclides in the composition of various chemical compounds whose biological properties differ sharply from the nuclide used.

The radiopharmaceuticals introduced into the body should not contain toxic impurities or radioactive materials, which during the decay form long-lived daughter nuclides [54].

A prerequisite for successful radionuclide therapy is the provision of a high local radiation dose in tumor cells at a low dose in healthy tissues.

Radiopharmaceuticals for radionuclide therapy are labelled with relatively short-lived radionuclides, which are chosen concerning their nuclear-physical, physical, chemical and biological properties.

There are general radiochemical requirements that should be followed with any labeling method: the yield of the target product in the synthesis reaction of the labeled compound should be maximum since the cost of radionuclides makes a significant contribution to the total price of the radiopharmaceutical; the specific radioactivity of the compound should be as high as possible; labeling and purification methods should ensure high radiochemical purity - higher than the purity of the preparations for diagnosis; labeling methods should ensure the preservation of the properties of the carrier molecule; the labeling method should ensure good stability of the compound during storage, transportation and in the blood circulation; due to high levels of radioactivity, it is desirable that labeling and purification be done automatically or under remote control; to facilitate the introduction into clinical practice, the radionuclide must be cheap and commercially available.

It should be noted that these requirements are contradictory. For example, a high yield of a labeled product requires a certain reaction time, since there is no chemical reaction that occurs instantly. At the same time, it requires a high concentration of reagents, including radionuclides, and materials with a high concentration of radionuclide exposed to radiolysis. The risk of damage is high in the radiolysis of proteins, the effectiveness of which is determined by the integrity of the structure [55].

The choice of a physiologically active molecule suitable for introducing a radionuclide into it is determined by the following principles:

- Molecule - the carrier of the radionuclide must participate in the metabolic processes of both malignant and normal cells;
- The molecule must penetrate fairly freely inside the malignant cell through the damaged membrane of this cell;
- The molecule must firmly bind to the surface of the malignant cell;
- The molecule must bind to a specific monoclonal antibody or a specific receptor [55].

In conjunction with such a molecule, a radionuclide can safely penetrate a malignant cell and destroy it with its radiation.

For effective use of radiopharmaceuticals in radionuclide therapy, it is necessary that with its help it would be possible to create a high and selective concentration of the drug in the target intended for irradiation, to ensure long-term retention of the radiopharmaceutical in it, with minimal concentration in normal tissue.

The selection of an isotope with a suitable half-life is carried out taking into account the pharmacokinetics of the transporter molecule (vector), which is designed to deliver the radionuclide to the zone of interest. For therapeutic effects, the radiopharmaceutical must be in the tumor for a sufficient time so that the radiation can destroy the malignant cells. For this reason, a sufficiently large molecular weight molecule, for example, a DNA molecule capable of incorporating into the nucleus of a malignant cell, is used as a carrier of a radiation source. But such a molecule slowly and difficultly penetrates the tumor and for a long time is not excreted from it. An isotope with a half-life that is too short will decay even before the molecule carrying it penetrates the tumor and settles in it. Not only will the therapeutic effect not be achieved, possibly harmful exposure to healthy and radiation-sensitive tissues. The larger the molecular weight of the carrier molecule and the slower its pharmacokinetics, the greater the half-life of the radionuclide should be. Also, the long half-life is favorable for the radionuclide therapy of some oncological diseases, for example, long-lived nuclides giving an antitumor effect are quite tolerant to the bone marrow. However, the half-life should not be too long, since an excessively long half-life increases the amount of radiopharmaceutical that must be moved to the tumor to achieve therapeutic levels of activity, taking into account decay and excretion.

In nuclear medicine, radiopharmaceuticals based on complexes with radioactive metals have found widespread use, and the same ligand can be used both to obtain a diagnostic drug and a therapeutic one. Thus, a complex of zollendronic acid with ^{99}Tc is used to diagnose cancerous metastases in the skeleton, and a complex with ^{l88}Re is used for radionuclide therapy. Some isotopes can also be used simultaneously in diagnostics and therapy, for example ^{177}Lu.

When choosing the half-life of an isotope, they are guided not only by the molecular weight, dimensions, and topological characteristics of the transporter molecule but also by the size of the tumor formations. So, if the target is scattered cells, then the half-life is chosen in the range from several hours to several days. Significantly long half-lives of the isotope (one or several weeks) are necessary to achieve a significant effect on solid tumors. Currently, considerations of logistics, cost and availability recommend the use of fairly short-lived therapeutic radionuclides for labeling low molecular weight proteins and peptides with fast clearance.

1.4.1 Radiopharmaceuticals labeled with metal isotops

In the composition of the radiopharmaceutical, radioactive isotopes of metals are usually used in the form of complex compounds. It is important that these complexes are stronger than complexes formed by certain proteins existing in the blood with the same element. Otherwise, when entering the body, the radiopharmaceutical will be quickly destroyed. For this reason, complexes of trivalent metals (REE, Ac, Bi, etc.) with bifunctional chelators are actively used in nuclear medicine. In general, the complex molecule can be divided into three different parts (figure 6):

1. biofunctional chelator (BFC)
2. linker between biomolecule and chelator
3. a biomolecule, for example, peptides or antibodies that play the role of an analog (key) for receptors (lock) expressed on the surface of cells.

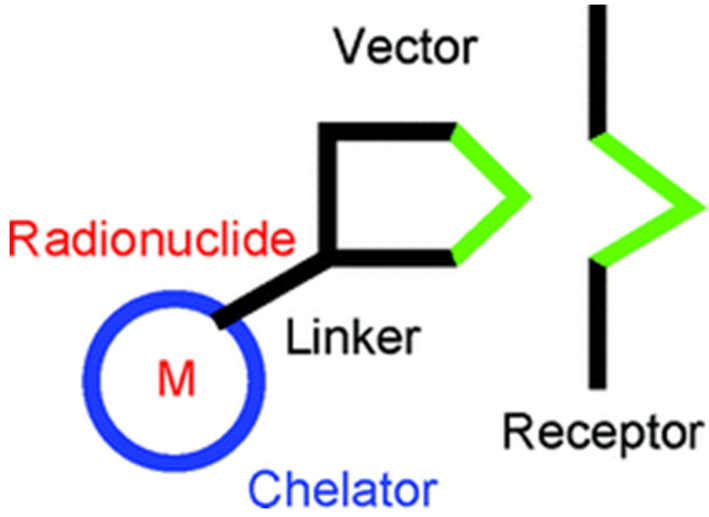


Figure 6 - The principle of interaction of the BFC and receptor [56]

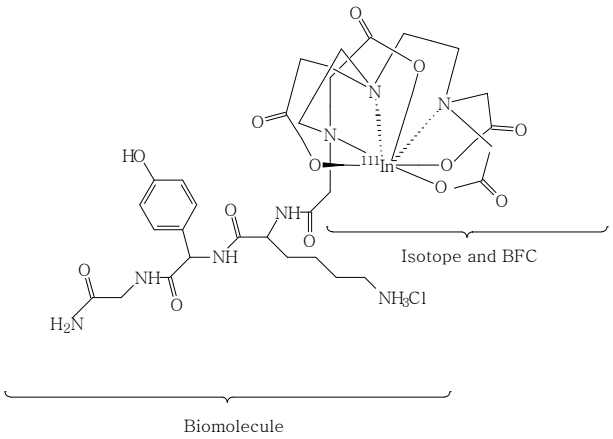


Figure 7 - Capromab Pendetide Radiolabeled with Indium-111 (ProstaScint)

Indium Pendetide Capromab (figure 7) is used to determine the degree of prostate cancer. Kapromab is a murine monoclonal antibody that recognizes a protein found in both prostate cancer cells and normal prostate tissue [57].

Some drugs used for radionuclide therapy are listed in table 2. Small inorganic complexes, peptides, and vitamins are used as carrier molecules. As bifunctional chelators, molecules, such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), which form strong complexes with a metal ion, is covalently linked to a targeting biomolecule.

Table 2 – RP in targeted therapy

Carrier	Radiopharmaceutical	Destination
Colloid	^{188}Re-Sn-FHMA	Rheumatoid arthritis, hemophilic disease[58]
Peptide	^{99}Y-DOTATOC	Neuroendocrine tumors[59]
Organicmolecule	^{131}I-MIBG	Pheochromocytoma and Paraganglioma[60]
Protein	^{188}Re-HA	Hepatocellular carcinoma[61]
Inorganicmolecule	Na^{131}I	Malignant and benign thyroid gland [62]
Steroid	^{125}I-tamoxifen	For cells containing estrogen receptors [63]
Nucleoside	5-[^{123}I] iododeoxyuridin	In an early ascites tumor [64]

1.4.2 Radionuclides

Particular attention is paid to the development of radiopharmaceutical drugs, with the help of which unbearable pain is relieved or, to a lesser extent, its severity is reduced. Basically, patients with bone metastases of various malignant neoplasms experience great pain. Analgesics and even conduction blockages cannot be effective in relieving pain [55].

The choice of a radionuclide is determined by such factors as the type of decay, half-life, radiation energy (i.e. the mean free path of particles in the tissue, LET and relative biological efficiency, RBE), specific activity, the natural prevalence of the mother nuclide, and radiochemical purity. The tendency of the selected isotope to separate in vivo from the carrier molecule is also taken into account. The radionuclide should emit corpuscular radiation (the ratio of non-penetrating radiation to penetrating radiation should be large). The maternal isotope from which the radionuclide is obtained must be stable, and the daughter is short-lived. Isotopes should have the required biochemical reactivity [55].

Table 3 presents the lanthanide radionuclides used in nuclear medicine, which are used in various types of therapeutic effects.

Table 3 - Examples of lanthanides used in nuclear medicine

^{47}Sc	$^{47}Ti\ (n,p)^{47}Sc$ $^{46}Ca\ (n,\gamma)^{47}Ca \rightarrow ^{47}Sc$	$T_{1/2}$=80.4 h	β⁻therapy / SPECT [65]
^{90}Y	$^{90}Sr/^{90}Y$ generator $^{235}U\ (n,f)^{90}\ Sr \rightarrow\ ^{90}Y$ $^{89}Y(n,\gamma) \rightarrow ^{90}Y$	$T_{1/2}$=64.0 h	β⁻therapy [66]
^{149}Tb	$^{152}Gd\ (p,\ 4n)^{149}Tb$ $^{142}Nd(^{12}C,\ 5n)^{149}Dy \rightarrow ^{149}Tb$	$T_{1/2}$=4.12 h	α-therapy /PET [67]
^{161}Tb	$^{160}Gd\ (n,\ \gamma)^{161}Gd \rightarrow ^{161}Tb$	$T_{1/2}$=165 h	β⁻therapy / Auger electron therapy / SPECT [68]
^{177}Lu	$^{176}Lu\ (n,\ \gamma)^{177}Lu$ $^{176}Yb\ (n,\ \gamma)^{177}Yb \rightarrow ^{177}Lu$	$T_{1/2}$=159 h	β⁻therapy / SPECT [69]
^{153}Sm	$^{152}Sm(n,\ \gamma)^{153}Sm$	$T_{1/2}$=45.6 h	β⁻therapy [70]

1.5 Chelators and linkers

In radiopharmaceuticals, radioactive metals bind to proteins or peptides and other classes of compounds. This is usually accomplished by using chelators, multidentate ligands, which form a non-covalent bond with the metal. The chelator intended for the introduction of the label must be bifunctional, i.e., contain one functional group for chelation, and another for adherence to the functional groups of proteins and peptides [71]. There are two ways to use chelators: pre-marking and post-marking. Postmarking consists in attaching a chelator to a peptide or protein, followed by the introduction of a radioactive metal [72]. Typically, post-labeling provides nearly 100% tagging efficiency without additional product purification. In the pre-labeling, a complex of radioactive metal with a chelator is first formed, and then this complex is combined with the target protein. In this method, the product yield is less than in the first.

The formation of chelate complexes is a reversible process. In addition to the thermodynamic stability of the complex, its kinetic properties are important. More kinetically inert chelates have lower rates of both association and dissociation. They are more stable in vivo, although labeling requires more severe conditions, such as elevated temperatures [73]. The stability requirements are quite high, since many plasma proteins, for example, transferrin (a plasma protein that carries iron transport) or ceruloplasmin (a copper-containing protein) present in blood plasma) also has chelating properties, and they are constantly trying to «take» away RP radionuclide [74]. Since the concentration of natural proteins in the blood is much higher than the concentration of labeled protein, the stability of the bifunctional chelate complex should be several orders of magnitude higher than the stability of the complex of the same metal with plasma proteins. Different

groups of metals, have different chemical properties and require different chelators for stable labeling [75].

Polyaminopolycarboxylate chelators easily form complexes with lanthanides (such as Lu, Sm or Ho with Y. Two classes of such complexing agents are distinguished: macrocyclic and acyclic (figure 8) [72].

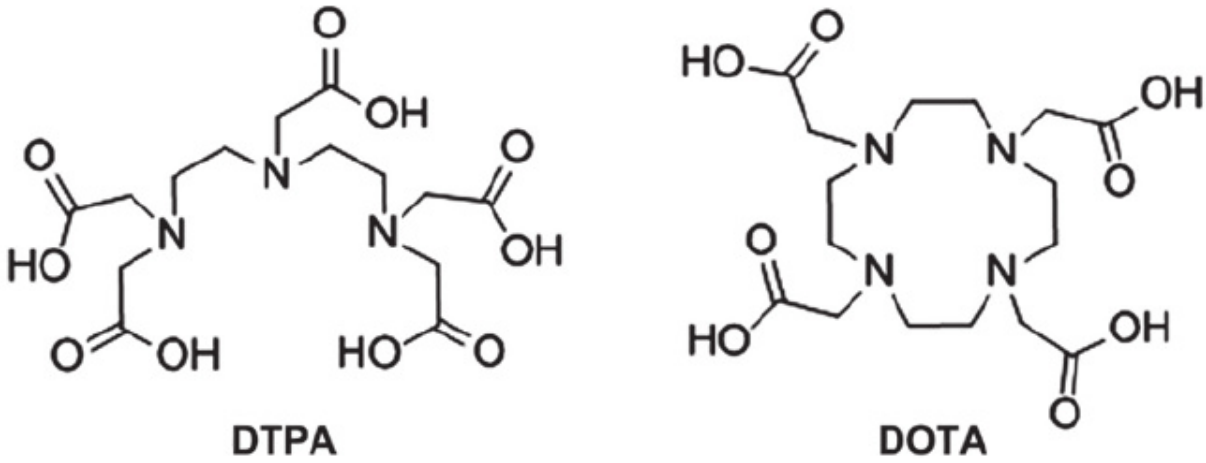


Figure 8 – Chelator Molecules

Commonly used macrocyclic chelators for radioactive lanthanides are various derivatives of DOTA. High kinetic inertness of the complex, i.e. the slow dissociation rate of DOTA, ensures the stability of communication with the radionuclide, however, due to the slow rate of association, an elevated temperature is required for labeling. DOTA derivatives are used for labeling short peptides, which are quite stable at temperatures of 60-90ºC [76]. Most acyclic polyaminopolycarboxylate chelators are various derivatives of DTPA (diethylene triaminopentaacetic acid). Complexes of various derivatives of DTPA with metals possess the required stability.

Although acyclic chelators are less inert, and therefore less stable than macrocyclic chelators, labeling is quick even at room temperature. For this reason, they are used to label monoclonal antibodies that cannot be heated [77]. There are techniques for attaching polyaminopolycarboxylate chelators to targeted proteins and peptides. There are two rhenium isotopes of interest for targeted therapy: ^{86}Re with average p-particle energy and ^{88}Re with high p-particle energy. Labeling with rhenium can be performed directly or indirectly. Direct labeling gives unreliable results and is generally not suitable for antibodies. The indirect method, which consists in creating a chelate complex, ^{88}Re with its subsequent attachment to the antibody, gives better results [78].

The linker is often used to modify the pharmacokinetic properties of a radiopharmaceutical. The linker can be, for example, a simple hydrocarbon chain to increase lipophilicity or a peptide sequence to improve hydrophilicity and renal clearance

(for example, polyglycine, polyserine, polyaspartic) [79]. Depending on the radionuclide and the bifunctional chelator, linker groups capable of rapid metabolism can increase the excretion of the radiopharmaceutical from the blood through the renal system. The choice of linker depends primarily on the pharmacokinetics of the requirements for radiopharmaceuticals. The final step involves conjugation between the BFC and the biomolecule, either directly or via a linker [79]. In principle, there are two approaches: the approach before and after labeling. In the last conjugation of BFC with a biomolecule, it occurs in front of the radioactive label with the corresponding radionuclide. The first step of radiolabeling is the first. The approach after labeling seems appropriate for biomolecules that are more resistant to the aggressive chemical environment during the chelation step, such as for peptides. More sensitive biomolecules are labeled with a preliminary label.

In the practice of combinatorial synthesis, the following linkers are most often used (figure 9):

- Chloromethyl (-CH2Cl),
- Hydroxyl (-OH),
- Amine ($-NH_2$),
- Aldehyde (-CHO),
- Silyl ($-OSiR_3$).

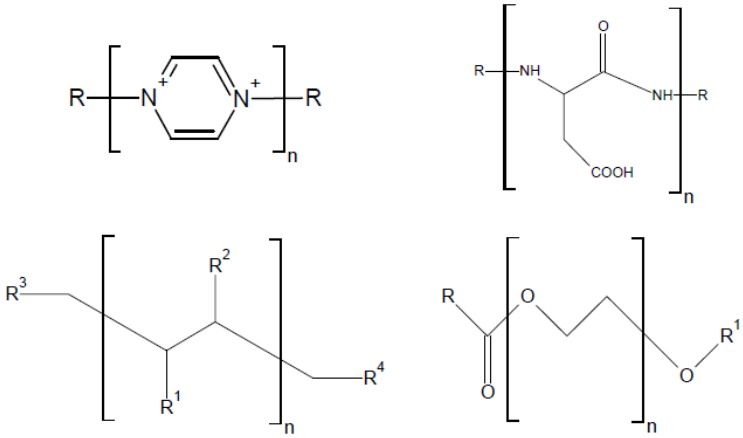


Figure 9 - Linker Examples

1.6 Selection of the main component

Thrice-negative breast cancer receptors are known to express gonadotropin-releasing hormone (GnRH) expression in more than 50% of cases [33]. Among the several GnRH analogues (agonists and antagonists) that have been studied, the non-peptide antagonist gonadotropin-releasing hormone 4 - [[(1R) -2- [5- (2-fluoro-3-methoxyphenyl) -3 - [[2-Fluoro-6- (trifluoromethyl) phenyl] methyl] -4-methyl-2,6-dioxopyrimidin -1-yl] -1-phenylethyl] amino] butanoic acid (elagolix (ELA). Based on this, elagolix can serve

a transporting agent for the delivery of a radioactive label of lutetium-177 in a tumor using it in theranostics.

To date, several works are known that describe the development of radiopharmaceuticals for the treatment of breast cancer of the type HER-2^{+}, while TNBC is characterized as HER-2^{-}. For example, drugs such as ^{177}Lu-DOTA-Trastuzumab are being developed [80],^{177}Lu-DOTA-F(ab$^{'}$)$_2$-trastuzumab [81], ^{177}Lu-DOTA-DN(PTX)-BN[82].

With this in mind, the main objective of the research is to develop a procedure for the preparation of a reagent based on elagolix, with an optimal shelf life and the possibility of its delivery to clinics. Because the elagolix molecule cannot be directly labelled with the lutetium-177 isotope, synthesis was performed at the University of Oslo, as a result of which the elagolix molecule was connected to the chelating molecule DOTA through an ethylene diamine bridge (figure 10).

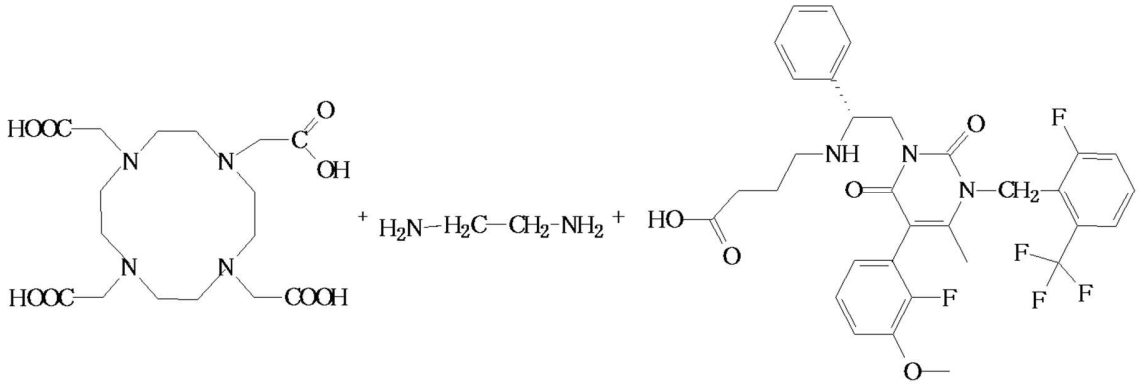


Figure 10 - Scheme of molecule synthesis for research

1.7 ^{177}Lu for medicine

^{177}Lu has a half-life of 6.89 days. When decaying, it emits β-particles with energies of 0.421 and 0.133 MeV, as well as gamma rays (γ) with energies of 208 and 113 keV. In nuclear medicine, β-particles are used for therapy and γ-rays are used for imaging. Lutetium-177 is the ideal radioactive isotope for simultaneous treatment and imaging. Such a possibility combined in one isotopeis called theranostic abilities [83].

In the indirect method, ^{177}Yb is formed by the (n, γ) reaction during neutron irradiation of a target enriched with^{176}Yb, transforms into ^{177}Yb with a half-life of 1.9 hours and then turns into ^{177}Lu as a result of decay. After radiochemical separation of ^{177}Lu from large amounts of the Yb target during neutron irradiation, it is possible to obtain^{177}Lu non-carrier with a specific radioactivity of up to 40.7 x 105 GBq / g [84]. Abundant^{177}Lu, has a high specific radioactivity and has increased value as an isotope for modern radioimmunotherapy of prostate cancer and breast cancer. Today, there is an increased demand for targets enriched in^{176}Yb to obtain the non-carrier isotope ^{177}Lu [85].

There are two alternative practical ways of producing ^{177}Lu, namely, the direct path (reaction 1), based on irradiation of the lutetium target with neutrons, and the indirect path, based on irradiation of the ytterbium target with neutrons, followed by radiochemical separation of ^{177}Lu from ytterbium isotopes. Both paths have been studied and discussed in recent years.

Direct route ^{177}Lu:

$$^{176}\text{Lu}\ (n,\gamma)\ ^{177}\text{Lu} \qquad \sigma = 2100\ \text{b} \tag{1}$$

This method is most preferred since ^{176}Lu has a relatively large reaction cross-section, ^{177}Lu can be obtained directly with high specific activity when neutrons are irradiated with ^{176}Lu in a nuclear reactor.

Due to the effective cross-sectional value, the activation reaction is higher than the calculated values. Upon irradiation of the ^{176}Lu target, as a result of a competing reaction, the isomer ^{176}Lu (n, γ) ^{177m}Lu is formed, which reduces the radionuclide purity of ^{177}Lu, preparation for preparation, and waste disposal in clinics. The natural mixture of ^{176}Lu isotopes contains only 2.6% [83].

Indirect way to obtain ^{177}Lu (reaction 2):

$$^{176}\text{Yb}\ (n,\gamma)\ ^{177}\text{Yb} \xrightarrow[1.9\ \text{h}]{\beta-} {}^{177}\text{Lu} \tag{2}$$

^{177}Lu can be obtained practically carrier-free with β decay of ^{177}Yb obtained by irradiation with ^{176}Yb neutrons in a reactor. The main requirement for improving the process is to reduce the formation of ^{169}Yb and ^{175}Yb isotopes from the enriched target. As a rule, it is possible to use the re-irradiated ^{176}Yb target, since its activation cross-section value is low and the target is practically not consumed. Upon receipt of a bearing-free ^{177}Lu with maximum specific activity, lutetium should be separated from the ytterbium target, since ytterbium easily forms complexes along with lutetium[85].

The theoretical activity resulting from the reaction (n, γ) during irradiation was calculated by the equation 3 [69, 86]:

$$A(Lu177) = \frac{N_0(Lu176) \times \sigma(Lu176) \times \varphi * k * \lambda(Lu177)}{\lambda(Lu177) + \varphi \times (\sigma(Lu177) - \sigma(Lu176) \times k)} \times$$

$$\left[e^{-\sigma(Lu176)\times k\times\varphi\times t} - e^{-(\lambda(Lu177)+\sigma(Lu177)\times\varphi\times t)}\right] \tag{3}$$

$$\text{With } k = G_{th} * g(T_n) + G_r * r(\alpha) * \sqrt{\frac{T_n}{T_0}} * S_0(\alpha) \quad (4)$$

where G_{th} and G_r are, respectively, the thermal and epithermal neutron self-shielding factors, $g(T_n)$ is the Westcott factor, $r(\alpha) * \sqrt{T_n T_0}$ is the spectral index, $S_0(\alpha) = S_o(Er) - \alpha$, where $S_0 = 1.67$ and E_r=0.158 KeV are constants of ^{176}Lu, and α is a measure of the epithermal flux deviation from the ideal 1/E distribution, where E is the neutron energy.

Recognized worldwide tool for calculating the neutronphysical characteristics of a reactor are numerical method based on the Monte Carlo method. Monte Carlo-based codes are routine for planning reactor irradiation. Evaluation of the produced activity by the equation (3) is rough and requires knowledge of some values that can be obtained using codes based on the Monte Carlo method. K-factor is a fitting parameter that can be determined from multiple experimental irradiations of lutetium.

1.8 Radiolabeling

Currently, a method is known for radiochemical labeling of the lutetium-177 isotope with the amino acid peptide ((Tyr3) -octreotate) associated with a bifunctional chelator tetraxetan (DOTA) (DOTA- (Tyr3) -octreotate, DOTATATE) by mixing a solution of lutetium-177 chloride with DOTATATE in composition with 0.5 M buffer solution of 4- (2-hydroxyethyl) -1-piperazineethanesulfonic acid (HEPES) with pH-4.0, at 90 ° C for 25 minutes. The resulting ^{177}Lu-DOTATATE complex was purified using a C18 Sep-Pak column, pretreated with methanol and water, followed by elution of the purified product with ethanol, which was removed by heating at 80 °C, followed by dissolution in sterile saline containing 1 mL 50 mg/mL sterile ascorbic acid solution and 4 mM ethylenetriaminepentaacetate (DTPA). The tagging efficiency is > 95% [87]. The disadvantage of this method is the use of toxic methanol compounds and HEPES buffer.

There is also known a method for radiochemical labeling of the lutetium-177 isotope with a humanized IgG1 antibody (Herceptin) bound to a bifunctional DOTA chelator (DOTA-Herceptin) by mixing a solution of $^{177}LuCl_3$ with DOTA-Herceptin in composition with 0.25 M ammonium acetate buffer solution with pH = 7.0, followed by adjusting the pH to 5 at 37 °C for 3 hours. The resulting ^{177}Lu-DOTA-Herceptin complex was purified by gel fit on a Sephadex G-25 column, followed by elution of the purified product with a phosphate buffer solution with the addition of 0,5% bovine serum albumin and subsequent addition of ethylene diamine tetraacetic acid (EDTA) to the latter to bind free lutetium. The efficiency of radiochemical labeling is 81% [35]. The disadvantage of the above method is the duration of the tagging process and the high consumption of reagents, as well as low tagging efficiency.

As shown by experiments, when applying the method described in the work [35], the yield of the labelled target product before filtration did not exceed 48% and in the filtrate not more than 3%. Most of the activity of ^{177}Lu remained unreacted or adsorbed

on the C-18 column. At the same time, an additional peak corresponding to the radiolysis product was detected on chromatograms.

Since there is a partial decomposition of the resulting complex, such a drug cannot be considered purify, safe and effective.

Therefore, the main objective of this study is to develop a procedure for producing the ^{177}Lu-DOTAELA complex, which would have an RCP level of at least 95%.

1.9 Purification

One of the most important problems in the synthesis of radiopharmaceuticals is the purification of impurities that reduce radiochemical purity. Impurities are the remains of the starting reagents, the products of adverse reactions. The methods chosen for the purification of labelled compounds depend on the chemical properties of the compounds, radioactive by-products and chemical impurities. The presence of radiochemical impurities in the radiopharmaceutical leads to excessive radiation exposure to the patient or to an undesirable high background of radioactivity, which reduces the contrast of the image or therapeutic efficacy. Therefore, if the process of radiolabeling leads to unsatisfactory radiochemical purity, then the purification step is inevitable.

There are various purification methods to increase the radiochemical purity of radiopharmaceuticals. The purification process requires in-depth knowledge of radiopharmaceuticals from structural features to susceptibility to various conditions. The main methods for purifying radiopharmaceuticals are solid-phase extraction (SPE), high-performance liquid chromatography (HPLC), size exclusion chromatography (SEC), ion-exchange chromatography (IEC), and liquid-liquid extraction (LCE).

There are two ways to introduce a radioactive label into a carrier molecule: direct and indirect.

With direct administration, the radionuclide is attached to the molecule via nucleophilic substitution and isotopic exchange. The most well-known examples of substitution and isotope exchange are 2-deoxy-2- [^{18}F], fluoro-D-glucose ([^{18}F] -FDG) and ^{131}I-metaiodobenzenguanidine ([^{131}I]-MIBG), respectively [109, 110]. Metal radionuclides can also be introduced directly. For example, peptides can be labelled with ^{99m}Tc via their thiol functional group [114].

Bifunctional chelating agents (BFCA) are usually used in the indirect method of introducing metal radionuclides. BFCA derivatives, such as diethylene triaminepentaacetic acid (DTPA), have been used to label various molecules with radionuclides, such as^{177}Lu [115] and ^{90}Y[116].

A common drawback of direct and indirect labeling methods is that none of them guarantees high radiochemical purity (RCP) of the final product [117]. Availability radiochemical impurities increase background radiation reduces specificity and exhibit increased radiation exposure [118]. In the case of diagnostic radiopharmaceuticals, radiochemical impurities can affect the interpretation of scans and the accuracy of the

image [119]. Impurities in therapeutic radiopharmaceuticals can also lead to side effects [120].

To achieve effective purification of the radiopharmaceutical, it is necessary to choose a more suitable purification method. The choice of method is based on consideration of the following criteria: molecular weight, lipophilicity, stability in the mobile phase, molecular charge [121-124] and the half-life of the radionuclide [125].

In the present work, solid-phase extraction was chosen as a method of purification of the obtained complex after radiosynthesis.

Solid-phase extraction is a simple, fast and powerful process that can separate dissolved or suspended compounds from other compounds in a mixture based on their physicochemical properties. This method was used for the synthesis, purification and concentration of radiopharmaceuticals [126]. Like other methods of liquid chromatography, it has a mobile and stationary phase. Based on the characteristics, the connection can be delayed in the stationary phase or pass through it.

The standard procedure for cartridges is as follows: load the reaction mixture into a column, remove impurities using water, and then elute the product with a suitable lipophilic eluent. Purification by solid-phase extraction from the cartridge occurs when the cartridge retains most of the product and impurities freely leave the column or vice versa. Parameters such as the type of cartridge, cartridge conditioning, sample concentration, flow rate and eluent lipophilicity affect the efficiency of purification steps [127]. Different purification procedures lead to different results. For example, ^{68}Ga-NOTA-ubiquicidin fragments (^{68}Ga-NOTA-UBI29-41, ^{68}Ga-NOTA-UBI30-41 and ^{68}Ga-DOTA-TATE) were purified using Strata ™ -X, C18-E light, Sep-Pak C18, which led to slightly changing values of radiochemical purity [128].

For a DOTA-like chelator-modified peptide labelled with $^{68}Ga^{3+}$, the main radiochemical impurity is free $^{68}Ga^{3+}$, which can be removed using Sep-Pak cartridges [129]. This method was used to separate free $^{68}Ga^{3+}$ and ^{68}Ga-DOTATOC, ^{66}Ga-DOTA-Tyr3-octreotide and ^{66}Ga-DOTA-biotin [130, 131]. A similar purification procedure was used for ^{68}Ga, ^{111}In, ^{177}Lu and ^{90}Y-labelled somatostatin analogues [132].

1.10 Chapter Summary

- TNBC is the most aggressive type of breast cancer, with a high probability of recurrence during the first years after surgery, as well as the formation of metastases and a decrease in life expectancy and quality of life, lack of expression of estrogen, progesterone and HER-2 receptors. Until recently, it was believed that TNBC cells do not have or have very few receptors on their surface. It has been found that gonadotropin-releasing hormone (GnRH) is expressed in more than 50% of cases by TNBC cells receptors. Therefore, the use of GnRH analogs will allow transporting the radionuclide to the tumor cells and conduct targeted radionuclide therapy.

- The mechanism of action of GnRH agonists includes two phases: a short-term stimulation phase and a pituitary desensitization phase when the gonadotrophs remain resistant to stimulation and the level of gonadotropins in the blood decreases. GnRH antagonists are opposite in nature and mechanism of action to agonists. After intravenous injection of GnRH antagonists, GnRH receptors are competitively blocked in the pituitary gland. Unlike GnRH agonists, antagonists act immediately and strongly bind to the GnRH receptor without causing its activation. Among GnRH analogues, nonpeptide antagonists are of particular interest. Of particular interest is the fact that these representatives have a lower molecular weight compared with GnRH agonists and peptide antagonists, which in turn affects the clearance of the compound in the body. For this reason, we were attracted by the analogue of the gonadotropin-releasing hormone elagolix.

- Breast cancer therapy can be carried out with one or a combination of methods such as: hormonal therapy, surgery, chemotherapy and radiation therapy. The negative effects on healthy organs limit the effectiveness of chemotherapy / radiation therapy. In order to reduce the negative consequences, it is necessary to apply more effective therapy. Specific drugs that are associated with biochemical processes that allow the differentiation of tumor tissue samples from healthy tissue. The required selectivity can be achieved by developing targeted radiopharmaceutical therapy.

- The development of radiopharmaceuticals obtained using radiometals for radioactive labeling or incorporation of a radioactive isotope into a carrier molecule is a lengthy and complex process. There are many requirements for radiopharmaceuticals for therapy and diagnosis. Radiopharmaceuticals should have high radiochemical purity and a high therapeutic dose, and the synthesis should preferably be simple and should not take much time. In addition, highly qualified personnel are required, exact observance of the radio-labeling procedure in accordance with good manufacturing practice (GMP), compliance with the requirements of the Pharmacopoeia and radiation safety. Based on the presented analysis, the following components were selected for the development of the radiopharmaceutical: Elagolix; DOTA chelating agent and ethylenediamine as a linker.

- The high demand for short-lived lutetium-177 for conducting radionuclide therapy is due to its penetrating ability (about 2 mm), nuclear-physical and chemical properties, which make it possible to obtain various complex compounds used in medicine. In

addition, radiopharmaceuticals based on lutetium-177 are supposed to be used for diagnostics.

To date, such ^{177}Lu-labelled radiopharmaceuticals for TNBC are not produced in the world and are not used in clinical practice. Their development will open up broad prospects for targeted radionuclide therapy of triple-negative breast cancer.

2. MATERIALS AND METHODS

2.1 Characteristics of used materials, supplies and equipment

In the course of experimental studies, various materials and materials were used, which are presented in table 4. Table 5 presents the installations and radiometric equipment.

Table 4 - Characteristics of the materials and materials used

№	Name	GOST, TC, country, company	Qualification or Grade
1	2	3	4
1	DOTAELA	Oslo, Norway	AG
2	Lutetium oxide	Trace Sciences International, Canada	82.0%, enriched by ^{176}Lu, spectroscopic purity of 99.99%
3	Acetic acid	GOST 61-75	AG
4	Nitric acid	GOST 11125-84	AG
5	Sodium Acetate	GOST 199-78	AG
6	Sodiumhydr oxide	GOST 4328-77	AG
7	Sodium chloride	GOST 4333-77	AG
8	Scandium oxide	TC 48-4-417-87	AG
9	Hydrochloric Acid	GOST 3118-77	AG
10	Glycerol	GOST 6824-96	AG
11	Ammonium acetate	GOST 3117-78	AG
12	Methanol	Sigma Aldrich, Germany	AG
13	Acetonitrile	Merck, Germany	AG
14	Ethanol	Merck, Germany	AG
15	Water for injections	INST Farm, Russia	
16	Ascorbic acid	GOST 4815-84	AG
17	Trifluoroacetic Acid	Sigma Aldrich, France	AG
18	DTPA	Fluka, Japan	AG
19	EDTA	GOST 10652-73	AG
20	Salicylic Acid	GOST 624-70	AG
22	Potassium chromate	GOST 4459-75	AG

Table 4 Continued

1	2	3	4
24	Starch	GOST 10163-76	AG
25	Silver nitrate	GOST 1277-75	AG
26	Nitric acid	GOST 11125-84	AG
27	Certified reference material	CRM (7837-2000, 7875-2000, 7834-2000, 7873-2000, 7836-2000, 7877-2000, 7835-2000, 7927-2001, 7143-95,7238-96)	AG
28	Sep-Pac C18	Waters, Ireland	AG
29	SCX	Sigma Aldrich, Germany	AG
30	Dowex 50W-X-12	BIO-RAD, Germany	AG
31	Purolite WCA 100	Purolite, USA	AG
32	KU-2	GOST 20298-74	
33	Thermometer(100 ^{o}C)	GOST 215-73	
34	Chromatographiccamera20x20	GOST 28365-89	
35	Penicillin bottle with rubber stopper, 10 ml	GOST 19808-86, TC 38-6108-95	Type 1-1
36	Indicator paper, 0-14	Machereynagel, Germany	AG
37	Crimper	Zonesun, USA	
38	Decrimper	Zonesun, USA	
39	Volumetric flask for 25, 100, 200, 500, 1000 ml	GOST 1770-74	
40	Glass beaker, 500, 1000 ml	GOST 25336-82	
42	Automatic dispenser 2-20 µl	Sigma Aldrich, Eppendorf reference, Germany	AG
43	Automatic dispenser 20-200 µl	Lenpipet, Thermoscientific, Russia	AG
44	Automatic dispenser 100-1000 µl	Lenpipet, Thermoscientific, Russia	AG

Table 4 Continued

1	2	3	4
45	Micropipette tips	Lenpipet, Thermoscientific, Russia	AG
46	Lead containers КТ1-10, КТ1-15	GOST 16327-88	
47	1, 2, 5, 10, 50 ml glas spipette	GOST 29227-91	AG
48	Adhesive layerpolyethylene tape	GOST 20477-86	
49	Scissors	GOST P 51268-99	
50	Chromatographic paper FN1	Sartogosm, Russia	AG
51	Calibration Source Cs-137		
52	Calibration Source Eu-152		
53	Set of weights G-2-210	StateMeter, USSR	AG
54	HPLC column Shim-pack VP-ODS	Shimadzu, Japan	AG
55	GC column DB-WAX	Agilent, USA	AG
56	Tracing paper	GOST 892-89	
57	Filter paper	GOST 12026-76	
58	Aluminum caps	GOST R 51314-99	Type K2-20

Table 5 - Installations and radiometric equipment

№	View equipment, appliance, inventory	Purpose of equipment, device, inventory	Model and year release	Note
1	2	3	4	5
1.	Thermal neutron reactor	Target irradiation	WWR-K	
2.	“Hot” camera	Conducting synthesis, opening ampoules after irradiation	MIP 1-2HD, Comecer, Italy	
3.	Gamma spectrometer	Determination of radionuclide purity	Ortec, EGPC 30-185-R, 2009, USA	HPGe

Table 5 Continued

1	2	3	4	5
4.	Gas chromatograph	Quality control	Agilent Technologies 7890A GC System, 2011, USA	FID detector
5.	High Performance Liquid Chromatograph	Quality control	Agilent 1260, 2004, USA	UV detector, Radiodetector
6.	Gamma-scanner	Quality control	Scan-RAM RADIO-TLC, LabLogic, 2014, Russia	500-1000 V
7.	Inductively coupled plasma atomic emission spectrometer	Quality control	SpectroGenesis, 2010, Germany	
8.	Dose calibrator	Quality control	CRC-25R, 2007, USA	
9.	Cabinet with a laminar flow of sterile air	Quality control	HERAguard, 2005	
10.	Electronic analytical balance	Solution preparation	AB-54	До 51 г
11.	Ultrasonic bath	Solution preparation	Branson 5510, 2002, USA	Ultrasonic purity 50-60 Hz
12.	Sterilizer	Sterilization		
13.	Sealing aluminum caps	Packaging		
14.	Electric Stove / Magnetic Stirrer	Radiosynthesis	LMS-1003, 2011, Germany	Maximumtemperature 380ºC
15.	pH meter / ionomer	Solution preparation	C 931, PT-10, Consortnv, 2005, Belgium	Relative error of pH ± 0,002
16.	Filling device	Packing	«Thimotheo-LT»	
17.	LAL-test	Quality control	Charles River, Carleston, SC, USA	

2.2 Methods of preparation of the starting components and solutions

Solutions used in the synthesis

0.45 mg/mL $LuCl_3$ solution.

A flask with a capacity of 10 ml was added with a weighed portion of lutetium oxide enriched in lutetium-176 (82%, Lu_2O_3) 6.36 mg (accurately weighed) and dissolved in 0.1 M HCl. At the end of dissolution, the volume is brought to the mark of boiled distilled water. In the resulting solution, the concentration of lutetium is 0.28 mg/ml.

0.01 M hydrochloric acid solution.

In a flask with a capacity of 100 mL, concentrated 36% hydrogen chloride (with a density of 1.189 g / mL) was added 0.085 mL, the volume was brought to the mark with boiled distilled water. In the resulting solution, the concentration of hydrochloric acid is be 0.01M.

Sodium acetate buffer pH-4.5.

In a 500 mL volumetric flask, weighed a portion of 31.5 g of anhydrous sodium acetate, dissolved in distilled water, added 90 mL of acetic acid, adjusted the pH to 4.5 and adjusted the volume of the solution with distilled water to the mark.

A solution of ascorbic acid.

1.76 g of ascorbic acid (exact weighed) is added to a 100 mL flask and dissolved in boiled distilled water. After the volume of the solution is adjusted with the same water. The resulting solution have a concentration of ascorbic acid of 100 mmol/L.

100 mmol/L salicylic acid solution.

1.38 g of salicylic acid (exact weight) was added to a 100 mL flask and dissolved in boiled distilled water. After the volume of the solution is adjusted with the same water. The resulting solution have a concentration of ascorbic acid of 100 mmol/L.

100 mmol/L DTPA solution.

In a flask with a capacity of 100 mL make a weighed portion of diethylenetriaminepentaacetic acid weighing 393 mg, 10 mL of 0.5 M NaOH are dissolved. After dissolution, make up the mark with boiled distilled water and mix. The resulting solution have a DTPA concentration of 10 mmol/L.

0.5 M sodium hydroxide solution.

In a flask with a capacity of 250 mL, a weighed portion of granular sodium hydroxide is added, 5 g of mass are dissolved in boiled distilled water and mixed. The resulting solution have a sodium hydroxide concentration of 0.5M.

EDTA Solution.

In a flask with a capacity of 100 mL make a weighed portion of the 2-aqueous disodium salt of ethylenediaminetetraacetic acid weighing 372 mg, dissolved in boiled distilled water, adjusted to the mark and stirred. The resulting solution have an EDTA concentration of 10 mmol/L.

Preparation of solutions used in quality control

10% solution of ammonium acetate.

In a flask with a capacity of 500 mL make a weighed portion of ammonium acetate weighing 50 g, dissolved in boiled distilled water and stirred. The resulting solution have a concentration of ammonium acetate of 10%.

0.9% sodium chloride solution.

In a volumetric flask with a capacity of 1000 mL, a weighed portion of sodium chloride weighing 9 g is dissolved in a small amount of distilled water, the solution volume is adjusted to the mark and thoroughly mixed.

Citrate Buffer pH 5.0

Samples of citric acid weighing 20.1 g and 8.0 g of sodium hydroxide are dissolved in water and the volume of the solution is adjusted with water to 1000.0 mL. The pH was adjusted to 5.0 potentiometrically with hydrochloric acid.

4% trifluoroacetic acid solution.

Trifluoroacetic acid, 2.7 mL (1.489 g/mL), was added to a 100 mL flask, dissolved with distilled water and stirred. The resulting solution have a trifluoroacetic acid concentration of 4%.

0.1% trifluoroacetic acid solution.

A 4% solution of trifluoroacetic acid with a volume of 2.5 mL was added to a 100 mL flask, dissolved with distilled water and stirred. The resulting solution have a trifluoroacetic acid concentration of 0.1%.

0.1 g/L Sc standard solution.

153.37 mg 200 mg is added to a 1000 mL flask; 20 mL of concentrated nitric acid are added, dissolved in distilled water and stirred. The resulting solution have a concentration of 0.1 g/L Sc.

0.01 g/L Sc standard solution.

In a flask with a capacity of 1000 mL make 100 mL of 0.1 g/L of scandium solution and bring to the mark with distilled water and mix. The resulting solution have a concentration of 0.01 g/L Sc.

0.02 M potassium chromate solution.

In a flask with a capacity of 250 mL, a weighed portion of granular potassium chromate is added, 0.97 g of mass are dissolved in boiled distilled water and mixed. The resulting solution have a potassium chromate concentration of 0.02 M.

Preparation of standard solutions with a concentration of 0.1 mg/mL.

The preparation of standard solutions using CRM is carried out by the instructions for use attached to the passport of each sample. Damaged ampoules are not allowed for use. For breeding use water for injection and nitric or hydrochloric acid.

CRM of metals of iron, chromium, copper, nickel, lead, zinc, aluminium, barium, manganese, molybdenum and tin have a certified value (1.0 ± 1.0) mg/mL, therefore they are diluted ten times.

The contents of the ampoule are transferred to dry glass.

5.0 mL of CRM solution was taken from a glass with a glass pipette and transferred to a 50 cm3 volumetric flask. Bring the volume to the mark with 1M or 3M solution of

nitric or hydrochloric acid. Mix the solution and pour into a plastic jar. A label is attached to the jar indicating the name of the element, the concentration of the element, the concentration of the background, the date of manufacture and the shelf life of the standard solution.

The CRM of arsenic has a certified value (0.0999 ± 0.8) mg/mL, the CRM of antimony is (0.1005 ± 0.4) mg/mL, so they are not diluted.

Preparation of 7 standard metal solutions with a concentration of 0.1 mg/cm^3

Solution No. 1. In a volumetric flask with a volume of 50 mL, 5 mL of solutions of CRM nickel are added, the solution volume is adjusted to the mark with a 1 M solution of nitric acid and mixed. The content of elements in the solution is 0.1 mg/mL.

Solution No. 2.5 mL of CRM solutions of iron, copper, aluminum, zinc is introduced into a 50 mL volumetric flask, the solution volume is adjusted to the mark with a 1 M solution of nitric acid and mixed. The content of elements in the solution is 0.1 mg/mL.

Solution No. 3. In a volumetric flask with a volume of 50 mL, 5 mL of a solution of CRM chromium are added, the volume of the solution is adjusted to a mark with a 1 M solution of nitric acid and mixed. The element content in the solution is 0.1 mg/mL.

Solution No. 4. The arsenic CRM solution is transferred to a plastic jar withoutdilution. The element content in the solution is 0.1 mg/mL.

Solution No. 5. In a volumetric flask with a volume of 50 mL, add 5 mL of a solution of CRM lead, bring the volume with a 1 M solution of nitric acid to the mark and mix. The element content in the solution is 0.1 mg/mL.

Solution No. 6. In a volumetric flask with a volume of 50 mL, add 5 mL of a solution of CRM tin, bring the volume with a 3 M solution of nitric acid to the mark and mix. The element content in the solution is 0.1 mg/mL.

Solution No. 7. In a volumetric flask with a volume of 50 mL, add 5 mL of a solution of CRM manganese, bring the volume with a 1 M solution of nitric acid to the mark and mix. The element content in the solution is 0.1 mg/mL.

2.3 Method of radiometric measurements

When researching the dissertation, radiometric measurements of the volumetric activity of the radionuclide of lutetium-177 bulk solutions obtained after dissolving the irradiated target using a dose calibrator were performed (figure 11).

Radiometers of this type are used in laboratories of radioisotope diagnostics and on the production sites of radiopharmaceuticals.

Figure 11 - Capintec CRC - 25R Dose Calibrator

The determination of volumetric activity (equation 5) is based on the following principle: a radioactive material has an initial activity of A_0 in a volume of V_0. Therefore, the concentration of activity in the solution before use, Bq/ml.

$$C_0 = \frac{A_0}{V_0} \tag{5}$$

For measurements, test solution vials were placed in a dose calibrator chamber. The received instrument readings are issued in [GBq]. To increase accuracy, the samples were measured 3 times with the subsequent determination of the average value of the activity measurements.

To account for the decay time, equation 6 was used:

$$A(t) = A_{ave} * e^{-\lambda \Delta t} \tag{6}$$

where A_{ave} is the average activity, $\lambda = 0.004$ h^{-1} decay constant of lutetium-177, Δt is the time interval reduced to one common time.

2.4 The preparation procedure of radiochromatograms

The distribution of the lutetium-177 radioisotope over the entire length of the chromatogram is obtained using the Scan-RAMRADIO-TLC, LabLogic gamma-scanning device (figure 12).

Radio chromatograms are necessary for calculating the radiochemical yield of ^{177}Lu-DOTAELA and quality indicators such as radiochemical purity (RCP) and the percentage of radiochemical impurity, radiochemicalyield.

Figure 12 - Gamma scanner Scan-RAM RADIO-TLC, LabLogic.

The method for producing radiochromatograms is based on the method of paper chromatography. A test sample with a ^{177}Lu radioisotope in a volume of 5 µl is applied using an automatic pipette to a previously prepared chromatographic strip, departing from the edge of 20 mm — the start line. Then the chromatographic strip with a spot is dried to dryness and placed in a prepared chromatographic chamber. The mobile phase is preliminarily added to the bottom of the chamber; the height of the solution in the chamber should be no more than 1 cm from the start line.

After that, the chromatograms are removed from the chambers and dried to dryness. Wrap in a transparent adhesive tape and carry out radio chromatography on a gamma scanner. Before taking measurements, calibrate it.

Upon completion of the scan, the computer displays information at what distance from the start line the maximums of ^{177}Lu gamma radiation are reached and the percentage of the areas of activity peaks to the total activity of the radio chromatogram.

The yield percentage and RCP (B) of the complex were calculated by equation 7:

$$B = \frac{A(\text{labelled complex})_1}{A(impurities)_2 + A(\text{labelled complex})_1} * 100 \qquad (7)$$

where A_1 is the number of counts of the labelled complex and A_2 is the number of counts of impurities.

The results of measurements of radio chromatography are recorded in the protocol.

2.5 Methodology for measuring the activity of radionuclides and determination of radionuclide purity

Radionuclide purity was confirmed on an ORTEC GEM-25185 spectrometer (V = 110 cm^3). The resolution of the spectrometer in the energy region of 208 keV, the mainline of ^{177}Lu, was 0.8 keV. Measurement error 5%. The γ-spectrometer is pre-calibrated by resolution and efficiency with the Eu-152 isotope (RSGS).

An aliquot of 1-5 µl is placed on filter paper, 1 x 1 cm in size and glued with adhesive tape on both sides.

2.6 pH determination procedure

Determination of the hydrogen index (pH) is necessary to control the pH of the stock solutions and the developed radiopharmaceutical intended for intravenous administration.

There are two ways to determine the pH: using a pH paper and a pH meter. The use of pH indicator paper can shorten the analysis time, this is especially important when working with radioactive drugs, however, pH indicator paper should be checked using standard buffer solutions, showing the color change for each pH unit, and also remember that the pH value measured using pH paper is approximate.

For potentiometric determination of pH, a pH meter was used. Calibration was carried out using standard buffer solutions with a pH of 1.68; 4.01; 9.18.

2.7 Method determination of the inactive impurities

The control over the content of metal impurities in solutions was carried out with an accuracy of 10^{-4} weight. % on an inductively coupled plasma atomic emission spectrometer from Spectro Genesis using an additive method.

2.8 Determination of radiochemical purity (PC)

The chromatographic paper of the FN1 and Whatman No. 3 type with a total length of 15 cm using the ascending method and 19 cm descending method was used as the stationary phase. Five μL of the test solution was applied at the start line at a distance of 3 cm and 6 cm, respectively, from the beginning of the chromatographic strip; after application, the spots were dried. Then the strips were placed in a sealed chromatographic chamber, and one end of it was immersed in a solvent.

2.9 Determination of radiochemical purity (HPLC)

For the HPLC analysis, the Agilent 1260 HPLC system (USA) equipped with a Shim-pack VP-ODS column with a reverse C18 phase (5 μm, 4.6 × 250) was used. HPLC was performed using Agilent pump system in combination with UV and radiometric (Flow-RAM) detectors.

2.10 Method of determination of residual solvents (GC)

The analysis is performed on an Agilent 7890A gas chromatograph equipped with a FID (flame ionization detector) detector and a vapour-phase sampler. The following parameters are set in the Zone Temps on the paraphase sampler module: the temperature in the thermostat is 80 °C, in the loop - 90 °C, in the transport line - 110 °C. The total time of one analysis is 6 minutes. 10 ml vials are installed in the sampler. The injection port is configured for split-injection of the sample in a ratio of 20: 1 and at 250 °C. When dividing, the DB-WAX column is used (30 m x 0.320 mm x 0.2 5μm). The temperature of the thermostat increases in stages: initially at 50 °C for 1 min, then it increases to 85

°C (at a rate of 20ºC/min) for 1 min. Helium is used as the carrier gas, with a column flow rate of 2.0 mL/min (Average speed: 42 cm/s). The hydrogen flow rate and airflow rates are 40 mL/min and 450 mL/min, respectively. The helium flow is set at a speed of 45 mL/min. The temperature of the detector is 250 °C.

1 μL of each sample is placed in a vial, thermostatically controlled 2 min, a vapour-phase sampler. Next, the steam-gas mixture is automatically introduced through the metering loop into the sample evaporator, where 20: 1 flow division occurs, then the sample falls into the chromatographic column where it is divided into individual components and then at the outlet of the column they enter the detector. On the chromatogram, we identify the components according to the retention time, measure the height or area of the peaks.

2.11 Procedure of radiolabeling

Eppendorf tubes were used to carry out the labeling reaction in 2 mL. In the case of using an automatic labeling system, 5-10 mL tubes were used as the initial ones, and the product was collected in 2 mL tubes.

2.12 Metrological processing of experimental data

To assess the reliability of experimental data, all results of radiometric measurements must be subjected to statistical processing. Processing of the obtained data can be carried out using Student's test in a confidence interval of ± 1% and with a confidence level of 95% of the average value. At the same time, radiometric measurements should be carried out for at least 5 repetitions, each of which should include successive corrections for the decay time of the radionuclide [88, 89].

The experimental data obtained during the research are processed using the least-squares method. For this, a scatter plot is constructed based on observational data according to equation 8 (x_i, y_i, i = 1; n) [90].

$$y = f(x, a_0, a_1, \dots a_k) \quad (8)$$

A straight line is selected that is closest to the points of the correlation field. According to the least squares method, the line is selected so that the sum of the squares of the vertical distances between the points of the correlation field and this line is minimal (figure 13) [91].

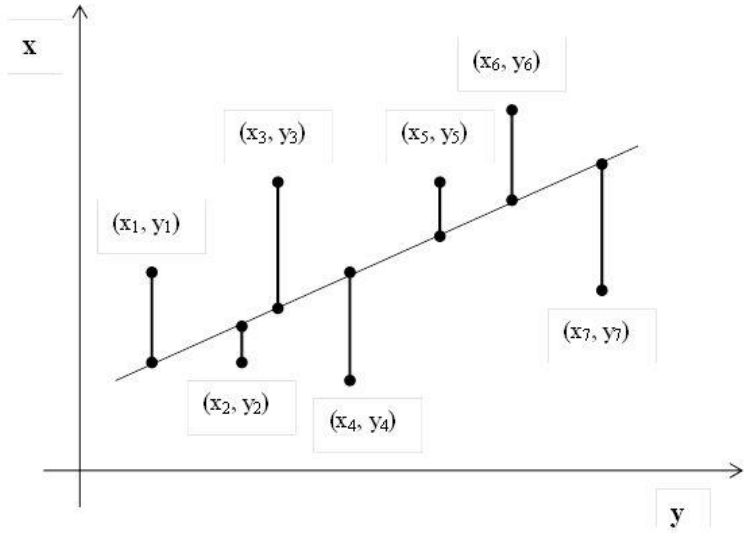


Figure 13 - Dependency graph y = f (x)[132]

Mathematically, this problem reduces to determining the parameters a0, a1, ... ak for which the function takes a minimum value.

Building a linear dependence of this function requires determining the parameters of the function y = ax + b. For this purpose, an equation is compiled for the function S by the equation 9 [91]:

$$S = \sum_{i=1}^{n} [y_i - ax_i - b]^2 \quad (9)$$

The values y_i and x_i = 1 ... n are known to us, these are observational data, in function S they are constants. The variables in this function are the desired parameter estimates - a and b.

The linear relationship between x and y is estimated using the linear pair correlation coefficient - $r_{x,y}$. It can be calculated by the equation 10 [93]:

$$\boldsymbol{r_{x,y}} = \frac{\frac{1}{n}\sum x_i y_i - \overline{xy}}{\sigma_x \sigma_y} \quad (10)$$

The range of acceptable values of the linear coefficient of pair correlation from –1 to +1. The sign of the correlation coefficient indicates the direction of communication. If $r_{x,\,y} > 0$, then the connection is direct; if $r_{x,y} < 0$, then the connection is inverse. If this coefficient modulo is close to unity, then the relationship between the signs can be interpreted as a fairly close linear one. If its modulus is equal to unity rx, y = 1, then the connection between the features is functional linear. If the signs x and y are linearly independent, then $r_{x,y}$is close to 0 [94].

To test the correlation coefficient, student t-test is used. This criterion is calculated by the equation 11 [95]:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \tag{11}$$

The obtained values of the Student coefficient were compared with the table with a confidence probability of 0.95 and the corresponding number of degrees of freedom. The correlation coefficient is substantially different from zero if the calculated value of t turns out to be larger than the tabulated value.

2.13 Chapter summary

1. The list and characteristics of materials, materials and basic equipment used in research on the development of a radiopharmaceutical based on DOTAELA labelled with lutetium are given.

2. Methods for preparing the starting components and solutions for the preparation of the developed radiopharmaceutical preparation are presented.

3. The methodology for radiometric measurements of the activity of gamma-emitting radionuclides in samples taken from the studied solutions of the future radiopharmaceutical is described.

4. A technique for producing radiochromatograms with a further determination of the radiochemical purity of the drug and the percentage of radiochemical impurities that may be in the prepared radiopharmaceutical is considered.

5. The methods used in quality control of the developed radiopharmaceutical are described.

6. The technique of statistical processing of the experimental results of radiometric measurements of ^{177}Lu activities in samples using Student's criterion is presented.

3. RESULTS AND DISCUSSIONS

3.1 Production of lutetium-177 with high and proper specific activity by neutron activation for further using in radiolabeling

The yield obtained directly by ^{177}Lu experimentally exceeded the theoretical value obtained at k = 1 by 180%. An individual characteristic of the reactor is the coefficient "k", which depends on the neutron spectrum. Based on the calculated and experimental values, the coefficient "k" is in the range of 1.5-2.5 [69, 96, 97].

The production of lutetium-177 at the WWR-K reactor was designed in two versions of the irradiation position, which is located (a) in the center of the reactor core and (b) on the periphery of the core. The design was performed using computer simulation using MCNP6 [98].

Lutetium-177 was produced at the WWR-K reactor by the direct method according to the reaction ^{176}Lu (n, γ) ^{177}Lu (see figure 14). Natural lutetium contains 97.41% Lu-175 and 2.59% Lu-176. To obtain a high specific activity of the target radionuclide, Lu-177, highly enriched (up to 82% in Lu-176) lutetium chloride was used.

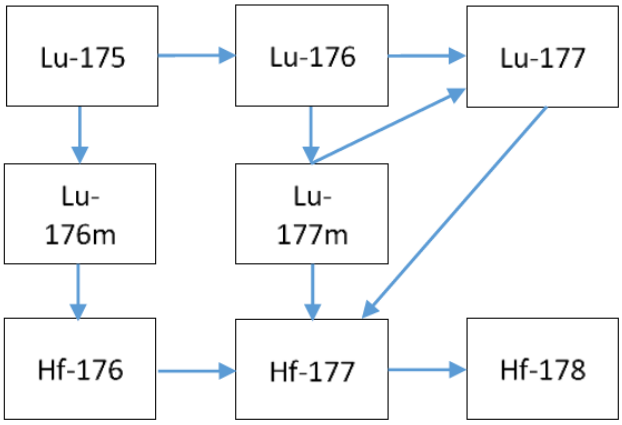


Figure 14 - Direct route for reactor production of Lu-177[99]

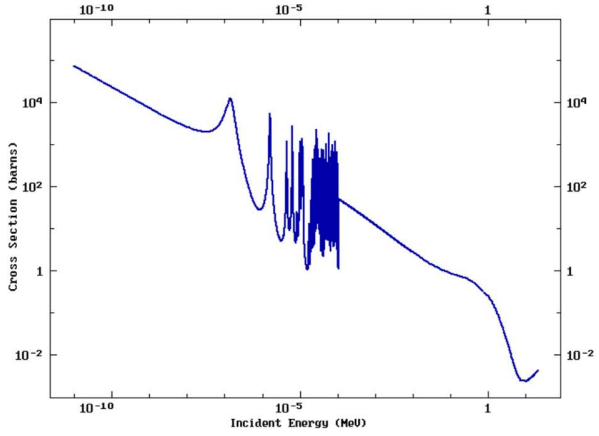
Lutetium chloride (mass of lutetium is 0.2 mg) is to be located in a sealed quartz ampoule, having outer diameter 8 mm (wall thickness is 0.5 mm), the height 35 mm and air environment inside. The quartz ampoule is in a sealed aluminium capsule, having outer diameter 26 mm, the height 80 mm and air inside. The aluminium capsule is installed in aregular irradiation device, cooled by primary circuit water.

The irradiation device was designed taking into account the following important factors:

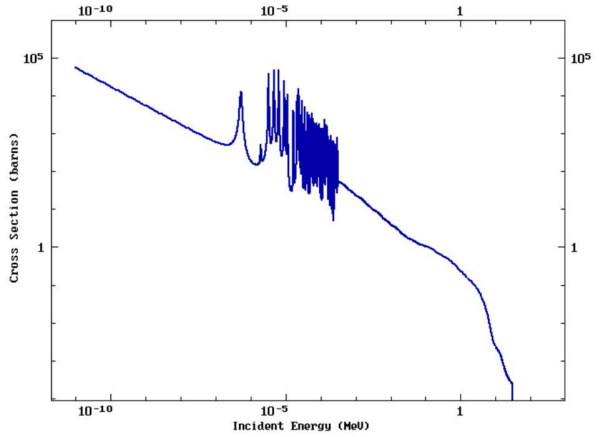
- selection of structural materials with a small neutron absorption cross-section;
- exclusion of interaction of the lutetium with reactor coolant;
- selection of the optimal mass, size and geometry of the target for reducing of self-shielding factor;

- lutetium capsule material should be convenient for the extraction of irradiated lutetium in a hot cell;
- the maximum possible preservation of the neutron moderator in the device.

Obtaining lutetium-177 by the direct method has more advantages than the indirect method of production since there is no need for radiochemical separation of lutetium-177 and a small amount of radioactive waste. The main disadvantage of this method is the presence of a parasite of the Lu-177m isotope having a long half-life (160.44 days). However, as is known [69, 100],its share of operating time in the process of irradiation is quite small (1-5%). The radiation capture cross sections for Lu-176 and Lu-177 are shown in figure 15[101]. Obviously, both sections do not obey the 1 / v law, in the region adjacent to the thermal region there are many resonances. Figure 15 (b) shows that the produced lutetium-177 has a large cross-section for radiation capture (about 1018 barns), which implies that its fading will affect the overall specific activity.



(a)



(b)

Figure 15 - The radiation capture cross-sections for: (a) Lu-176 and (b) Lu-177 [99]

Before carrying out experimental work on the irradiation of lutetium in a research reactor, preliminary calculations are carried out using computer codes aimed at solving the neutron transport equation by one or another method (deterministic approach, Monte

Carlo method, etc.). Solving unsolvable and complex problems using the Monte Carlo method, which is used for computer modeling of statistical processes based on a deterministic approach, is very useful.

In this work the transport code MCNP6 with nuclear data libraries ENDF/B-VII.1 [102] and JEFF-3.2 [103] was used. The program allows to take into account all the chains of nuclear transformations accompanying the formation of lutetium-177 [100]. The self-defense effect is an important factor that depends on the target geometry and prevails in the case of a target with a large activation cross section and a low target volume to surface ratio [96, 104].

The calculations were performed for the current state of the reactor core shown in figure 16. The calculations take into account the actual fuel burn up in fuel assemblies and poisoning in the annular beryllium reflector.

Modeling the irradiation device is important to correctly describe the geometry of the capsules and targets, the composition of the material. Using the cross section values from the Library for the materials used is also important.

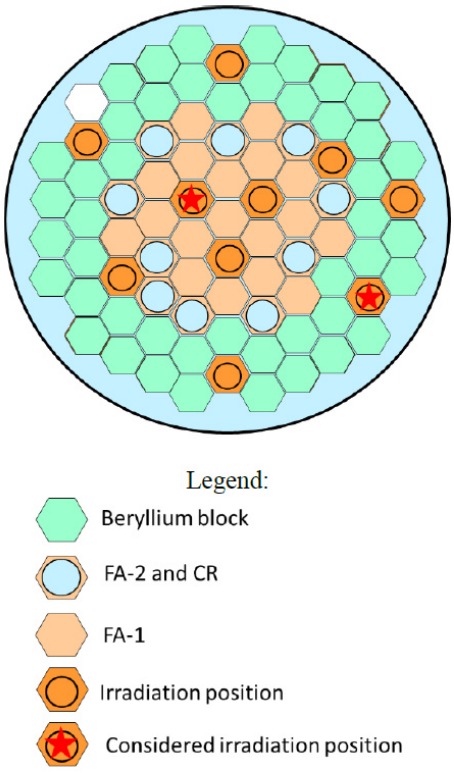


Figure 16 - WWR reactor core map [99]

It is quite obvious (figure 16) that the "softer" the neutron spectrum in the region of irradiation of the lutetium target, the more intense the reaction of capture of neutron radiation. The corresponding energy spectra of neutrons in the studied irradiation sites are shown in figure 17. The maximum activity can be obtained according to the conditions specified in table 6.

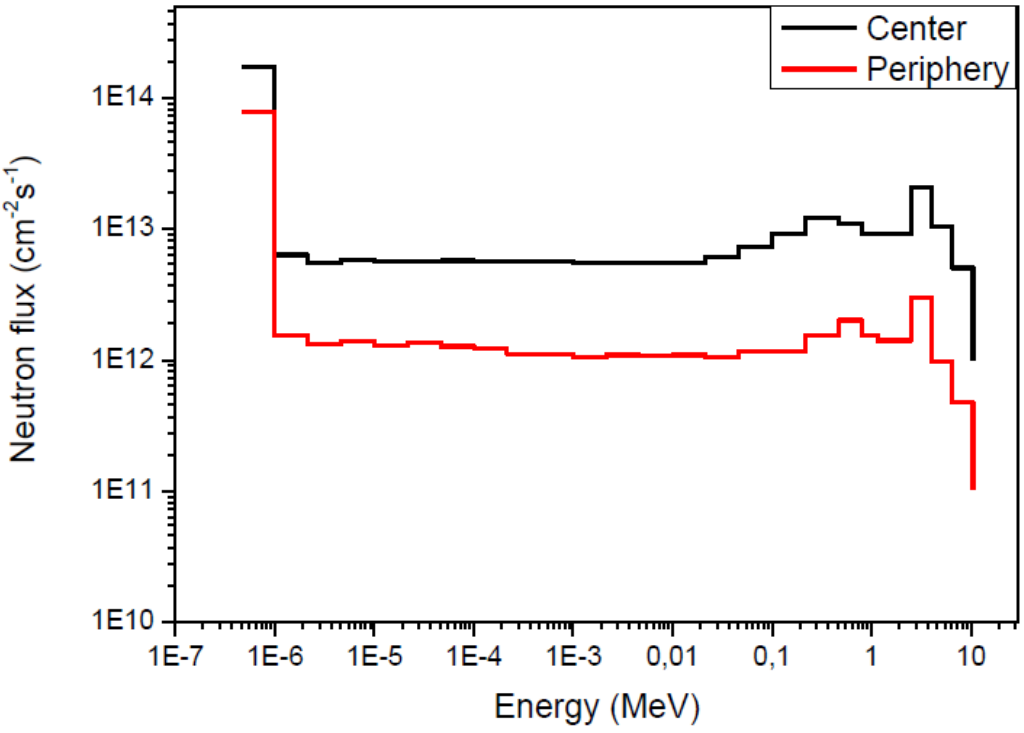


Figure 17 - Energy neutron spectra in the irradiation positions [99]

The theoretical calculation of the output of Lu-177 is presented in figure 18.

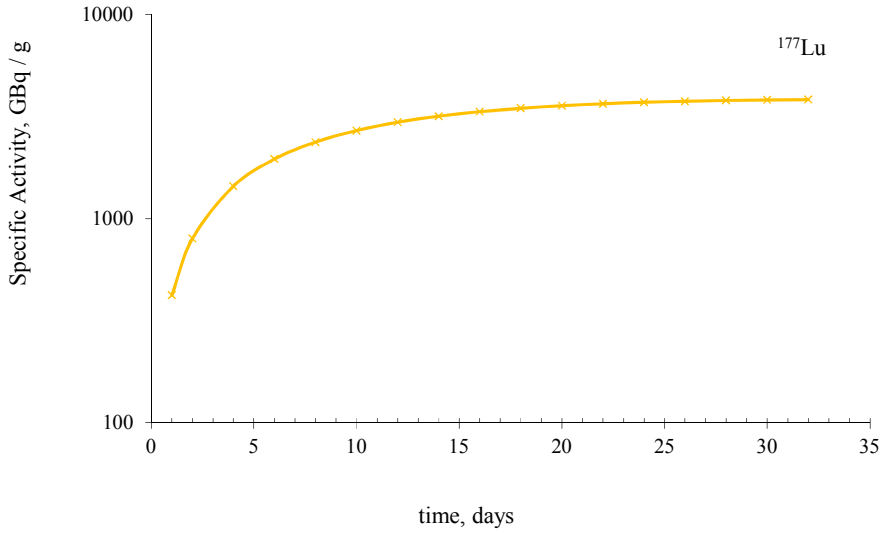


Figure 18 – Theoretical calculation of the output of Lu-177

Table 6 Irradiation conditions and a maximal produced activity ofLu-177[99]

Irradiation position	Core center	Core periphery
Thermal neutron flux(E_n<0.625 eV), $cm^{-2}s^{-1}$	$1.2x10^{14}$	$6.0x10^{13}$
Irradiation temperature,°C	50	50
Target mass of lutetium, mg	0,2	0,2
Effective irradiation time, days	15	20
Specific activity of Lu-177, GBq/mg	819	561

Lutetium mass variation versus irradiation time is given in figure 19.

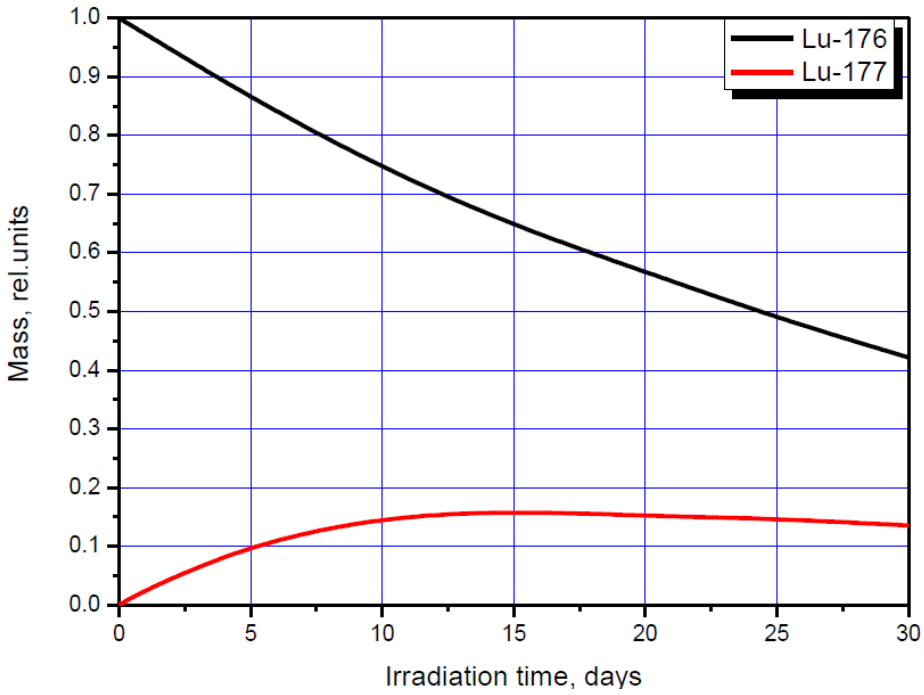


Figure 19 - The accumulated Lu-177 specific activity in considered irradiation positions [99]

The Lu-177^m content is mainly depending on two factors: irradiation and cooling time. Cross section of nuclear reaction $^{176}Lu(n,\gamma)^{177m}Lu$ is 2.8 barn and cross section of Lu-177m burn up reaction ($^{177m}Lu(n,\gamma)^{178}Lu$) is 626 barn [100]. Obviously, the activity of the produced Lu-177m will not be large. The predicted activity of Lu-177m will be in range 13.4-21.3 GBq (see figure 20).

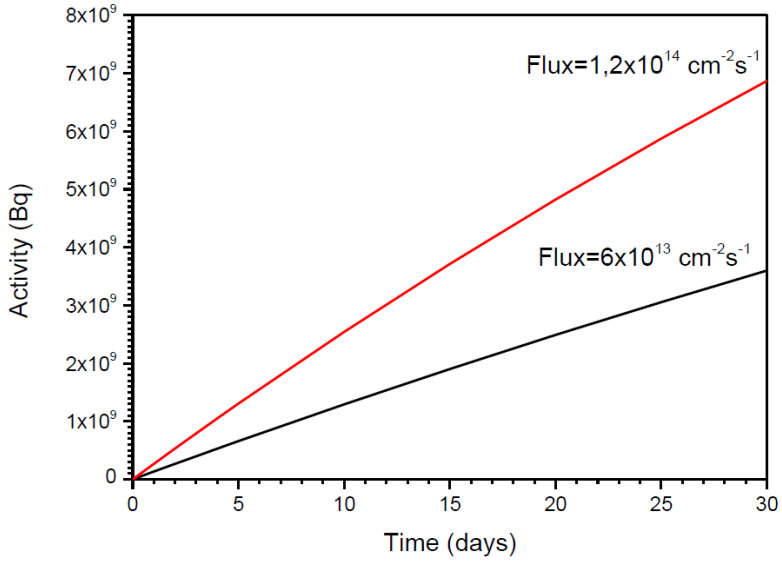


Figure 20 - Predicted activity of Lu-177m (k=1.5) [99]

Lutetium-177 is one of many promising radioisotopes for radionuclide therapy. In the Republic of Kazakhstan, the isotope of lutetium-177 can be obtained only at the WWR-K research reactor by the nuclear activation reaction (n, γ). The irradiation zone of the reactor target has many irradiation positions with different neutron fluxes in them. This study showed that 819 GBq / mg Lu-177 with specific activity as a result of a direct nuclear reaction can be obtained by irradiation in a WWR-K reactor with a thermal neutron flux of $1.2 \cdot 10^{14}$ $cm^{-2} \cdot s^{-1}$. Lu-177m is formed during irradiation and is a parasitic radioisotope during this irradiation time, and its activity is about 2.5% of that of Lu-177. Thus, the possibility of serial production of Lu-177 at the WWR-K reactor was demonstrated. One of the fundamental aspects is the specific activity of lutetium-177, which is a measure for evaluating its use in targeted radionuclide therapy.

The result of this work is recommendations for increasing the specific activity of lutetium-177:

- the use of a nuclear reaction where the target is ytterbium to produce lutetium-177 (indirect nuclear reaction). In the reference [97, 105] this method makes it possible to obtain a high specific activity of lutetium-177; a distinctive feature is the radiochemical separation of the target nuclide from the target material;
- to analyze the possibility of creating a neutron trap to increase the neutron flux and the fraction of thermal neutrons in the integral neutron flux.

3.2 Radiolabeling DOTAELA with produced lutetium-177 and determination the optimal synthesis of the DATOELA-^{177}Lu complex condition

3.2.1 Selection of mobile phase systems for chromatographic studies of DOTAELA labelled with isotope lutetium-177

A chromatographic system was developed for the DOTAELA compound. Radio-labeling was carried out with the lutetium-177 isotope obtained by irradiating 400 µg of lutetium-176-enriched chloride with a thermal neutron flux of 2 * 10^{14} n/cm^2 * s for 291 hours. After irradiation, the target was held for 6 hours and then in a hot chamber to open the ampoule, followed by the dissolution of the target in 2 mL of a 0.01 M hydrochloric acid solution.

An aliquot of the stock solution was selected 20 days after irradiation and diluted in 10 mL. Three samples (table 7) of 5 µL of the resulting solution were measured on a gamma spectrometer (ORTEC). An example of the obtained spectrum is shown in figure 21.

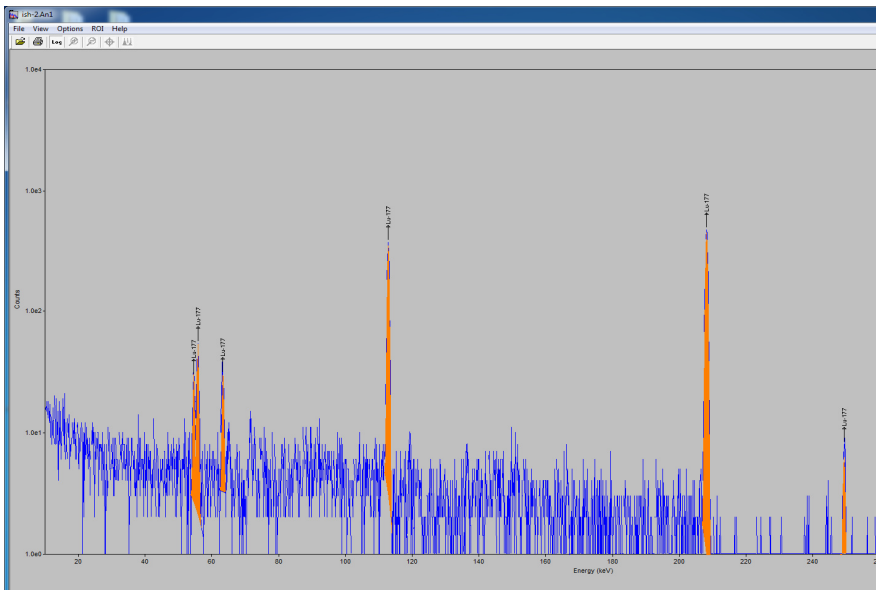


Figure 21 - Gamma Spectrum of Lu-177

Analysis of the obtained spectrum showed the absence of radionuclide impurities in samples of enriched lutetium after irradiation.

Table 7 - The measurement results of 3 samples of Lu-177

Sample Lu-177	Measurement time, s	Activity, Bq
1	600	1.35E+05
2	600	1.30E+05
3	600	1.38E+05
Averageactivity		1.34E+05
SE, %		3.2

For DOTAELA radiolabeling, 71 μL of DOTAELA solution was taken into a 10 mL vial so that the final concentration was 30 μg/mL, then 125 μL of an acetate buffer solution with pH 4.5 was added, then 8 μL of lutetium-177 chloride was added and the volume was adjusted to 2 mL. The resulting mixture was placed in a glycerin bath at 80-90 °C for 30 minutes. At the end of time, the bottle was removed and cooled. Then a sample was taken for chromatography.

The chromatographic paper of the FN1 and Whatman No. 3 type with a total length of 15 cm using the ascending method and 19 cm descending method was used as the stationary phase. Five μL of the test solution was applied at the start line at a distance of 3 cm and 6 cm, respectively, from the beginning of the chromatographic strip; after application, the spots were dried. Then the strips were placed in a sealed chromatographic chamber (figure 22), and one end of it was immersed in a solvent. The following phases were used as mobile phases: 10% solution of ammonium acetate in methanol (30:70 v / v); sodium chloride solution 0.9%; 0.1 M sodium citrate buffer, pH 5.0.

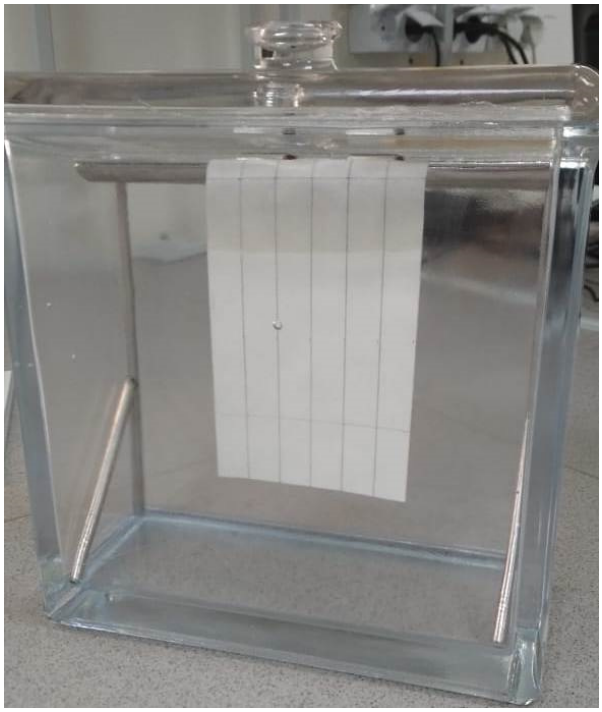


Figure 22 - Chromatographic chamber

In the course of the work, when analyzing the chromatograms, it was decided to use FN1 chromatographic paper in the solvent downward current system, due to the fact that the chromatography time is faster.

As shown by the experiments, the DOTAELA complex labelled with lutetium-177 does not dissolve in aqueous solutions. This fact is confirmed by the chromatograms shown in figures 23 and 24.

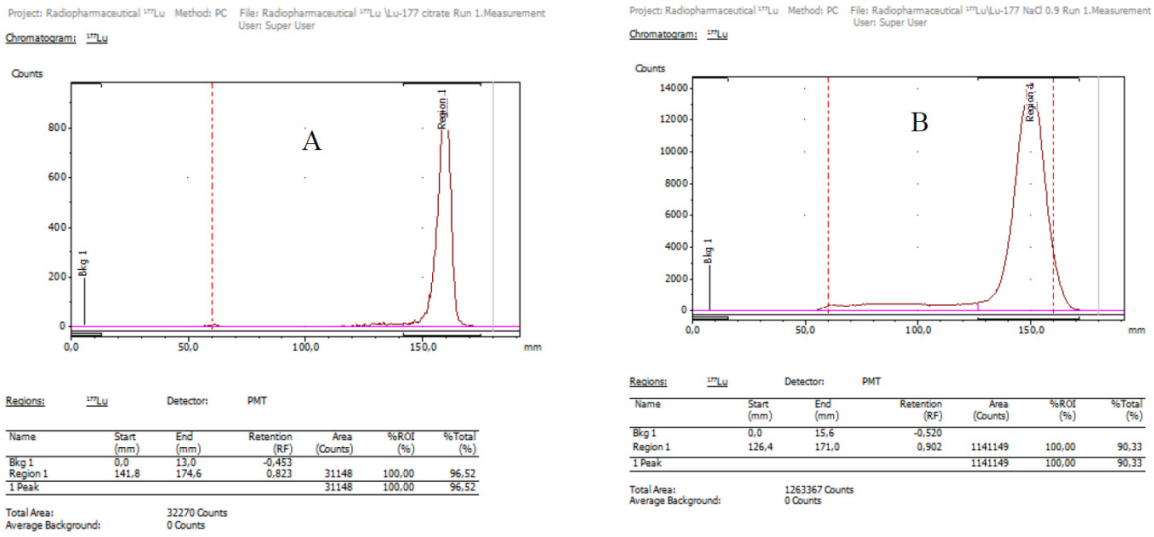


Figure 23 - Chromatograms (A and B) $^{177}Lu^{3+}$ [106]

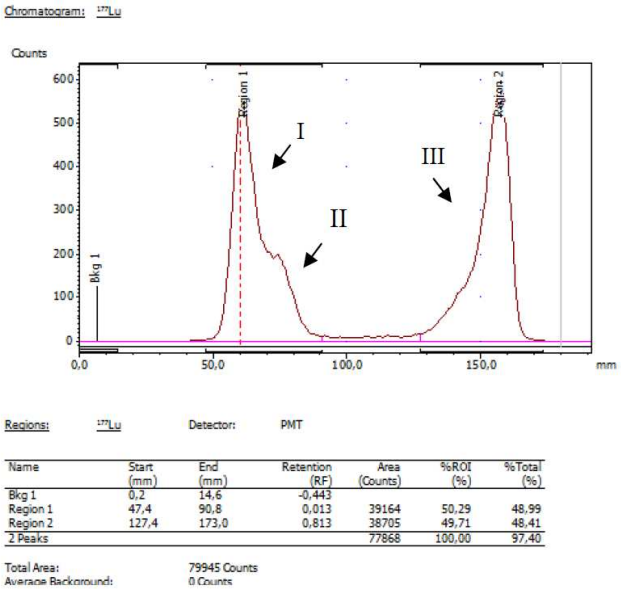


Figure 24 - Chromatogram of ^{177}Lu-DOTAELA. Peaks (I): ^{177}Lu-DOTAELA, (II): fragments of radiolysis ^{177}Lu-DOTAELA and (III): $^{177}Lu^{3+}$ [106]

In figures 23 and 24, the main peak ^{177}Lu-DOTAELA detected by a scintillation detector (NaI) after using an aqueous solution of sodium citrate as the mobile phase is located on the start line ($R_f = 0$), and the peak corresponding to free Lu-177 moves along with the chromatogram along with the front solvent ($R_f = 1$). It follows that ^{177}Lu-DOTAELA does not interfere with the determination of unreacted Lu-177.

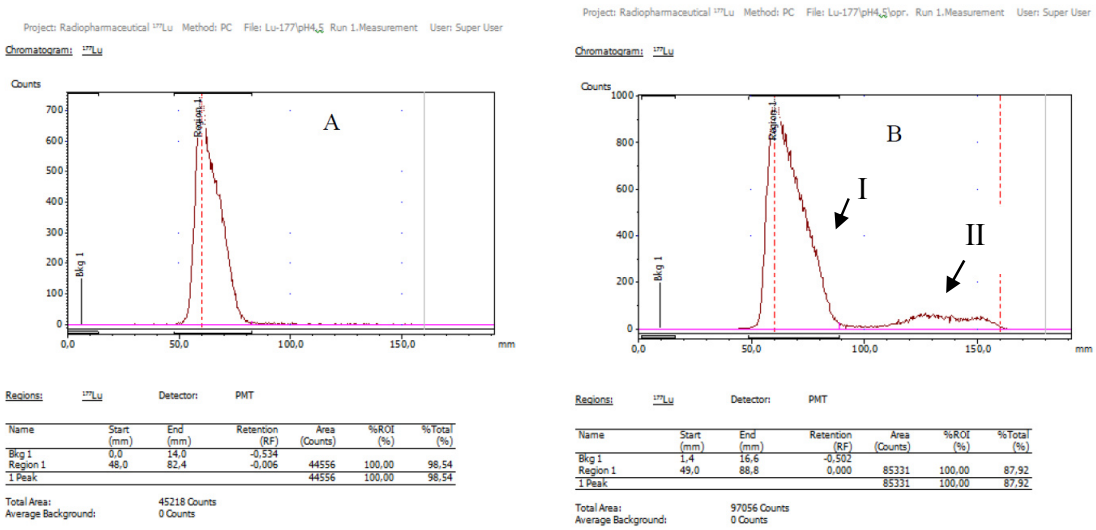


Figure 25 - Chromatograms: A- $^{177}Lu^{3+}$ и B- (I): $^{177}Lu^{3+}$ и (II) ^{177}Lu-DOTAELA[106]

To obtain the chromatograms in figure 25, a chromatographic strip was placed in a system of the organic mobile phase of ammonium acetate in methanol. After chromatography in an organic medium, analysis of the chromatogram in figure 25 showed that unreacted Lu-177 remains on the start line and does not move along with the solvent front, and compound^{177}Lu-DOTAELA, on the contrary, moves with the solvent front and does not have a clear peak character, therefore this system not suitable for separation.
A ^{177}Lu-DOTATOC chromatogram obtained in citrate buffer solution was used as a standard (figure 26).

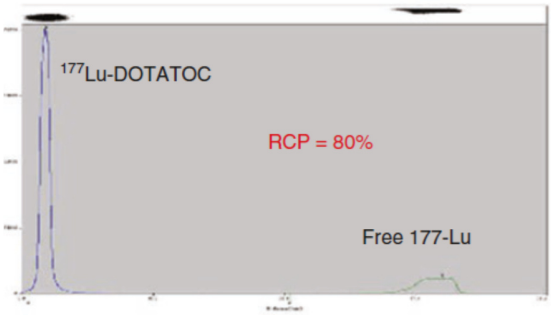


Figure 26 - Comparison standard for the determination of RCP in a citrate buffer system[107]

Thus, it was found that citrate buffer solution has the best parameters for studying the behavior of ^{177}Lu-DOTAELA and obtaining chromatograms of the products of its interaction with ^{177}Lu.

It should be noted that the use of chromatographic techniques for the synthesis products in two mobile phases of sodium chloride and a citrate buffer solution makes it possible to evaluate the radiochemical purity of the labelled target product - "^{177}Lu-DOTAELA".

The results obtained by HPLC are consistent with those obtained by paper chromatography. However, the capture of $^{177}Lu^{3+}$ ions in the reverse phase of an HPLC column does not provide the necessary reliability in the analysis of the radiochemical yield; therefore, in this case, it is preferable to use the method of paper chromatography, which gives an idea of the content of radiochemical forms of ^{177}Lu.

3.2.2 Determination of the optimal parameters of DOTAELA radio-labeling with the isotope lutetium -177

The lutetium-177 isotope was obtained by irradiating 400 μg of lutetium-enriched lutetium-176 (82.0%) lutetium chloride with a thermal neutron flux of 2 * 10^{14} n / cm^2*s for 240, 252, 408 hours. After irradiation, the target was held for 24- 36 hours after which ampoules were opened in a hot chamber and the targets were dissolved in 2 ml of a 0.01 M hydrochloric acid solution. The next step is quality control.

The specific activity of ^{177}Lu obtained after irradiation of the enriched target for different time durations and at different positions of the thermal neutron flux are given in table 8.

Table 8 – Irradiation parameters of ^{177}Lu

Irradiation time, h	Irradiation channel	Activity^{177}Lu, GBq (predicted)	Activity^{177}Lu, GBq (observed)	The specific activity of ^{177}Lu, GBq/mg
252	9	85.0	88.4± 3	442
408	10	106.3	152± 2	760
240	16	82.7	53.4± 3	267

The maximum specific activity was 760 GBq/mg, which was achieved by irradiation with a thermal neutron flux of 1-2 * 10^{14} n/cm^2 *s for 408 h. Table 8 shows that the data on irradiation of ^{177}Lu was significantly higher compared to theoretically calculated values taking into account thermal neutron capture only. A possible reason for obtaining high values of practical activity, in comparison with theoretically calculated values, can be explained by the contribution of epithermal neutrons, which are not taken into account in theoretical calculations [108-110].

Studies to determine the optimal parameters of the process of obtaining the ^{177}Lu-DOTAELA complex (figure 27) were carried out in the direction of studying the influence of such parameters as incubation time, temperature and pH to obtain the maximum ^{177}Lu-DOTAELA during complexation. Keeping the reaction volume of 2 mL, the amount of DOTAELA remained constant to determine the optimal parameters of time, temperature and pH to obtain maximum complexation. The assessment of the labelled conjugate and the yield of complexation were determined by paper chromatography in 1 M sodium citrate buffer solution, pH 5.0. The yield of ^{177}Lu-DOTAELA was evaluated using paper chromatography [106].

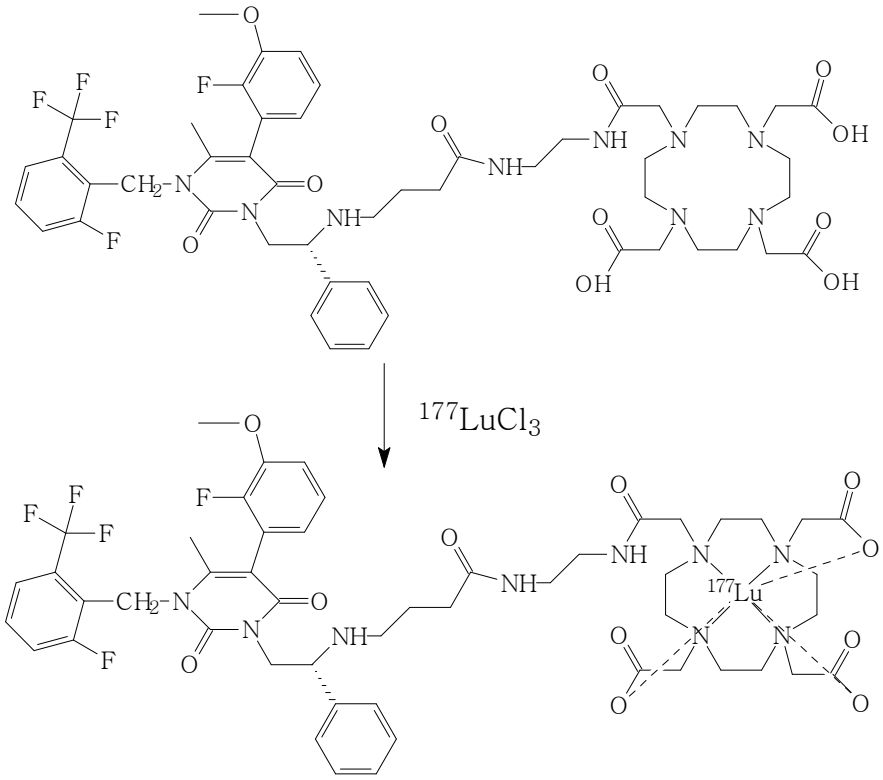


Figure 27 – Synthesis scheme of ^{177}Lu-DOTAELA

Paper chromatography studies were performed after passing through a 12 cm solvent front. For these purposes, 5-10 μL of the test solution was applied at a distance of 6 cm from the bottom of the paper strips, which were chromatographed in a citrate buffer solution with pH = 5.0. Then the strips were dried and then glued with adhesive tape on both sides. After that, the activity distribution on the strip was measured using a radio chromatogram scanner with a NaI detector. The radiochemical yield (B) of the complex is calculated by equation 12:

$$B = \frac{A(177LuDOTAELA)}{A_{177Lu}+A(177LuDOTAELA)} * 100 \quad (12)$$

The yield of ^{177}Lu-DOTAELA depending on pH, time, and temperature is shown in tables 9, 10 and figure 28, respectively.

Table 9 - Dependenceof^{177}Lu-DOTAELA yieldonpH

№	pH	Time, min	Temperature, °C	B, %
1	2.0	30	60	21.3 ± 0.5
2	4.5	30	60	50.1 ± 0.3
3	6.0	30	60	46.3 ± 0.7

The pH value plays an important role in increasing the rate of formation of complexes. Due to the fact that at pH = 6 and above, lanthanide cations form insoluble hydroxides, the optimum pH for radioactive labeling is the range between 2 and 6, which is usually achieved by using buffer solutions.

Eight μL of 0.01 M HCl solution with activity of Lu-177 2.6–2.7 GBq was added to a freshly prepared DOTAELA solution (71 μL) dissolved in ethanol and adjusted to pH 2-6 with an acetate buffer solution (125 μL), then the volume was adjusted with purified water to 2 mL.

According to the data obtained on the study of the influence of the pH of the synthesis medium on radiochemical purity, it was found that, with an increase in pH values from 2 to 4.5, the B value increases, and with an increase in pH to 6, the RCP value monotonously decreases. As a result, the optimum pH of the synthesis of the ^{177}Lu-DOTAELA complex is 4.5.

After determining the optimal pH, the reaction time mode was studied (5, 30, and 60 min), table 10.

The results indicate that the optimal time for complexation is 40 minutes, since a further increase in time practically does not affect the yield of the complex.

Table 10–Dependence of ^{177}Lu-DOTAELA yield on synthesis time

№	pH	Time, min	Temperature, °C	B, %
1	4.5	5	60	24.7 ± 0.7
2	4.5	30	60	50.1 ± 0.9
3	4.5	60	60	49.4 ± 0.8

Further, at a constant pH value of 4.5, the dependence of the B was studied at various modes of complexation temperatures (25, 40, 60, 80, and 100 °C, figure 28). The reaction was carried out for 40 minutes.

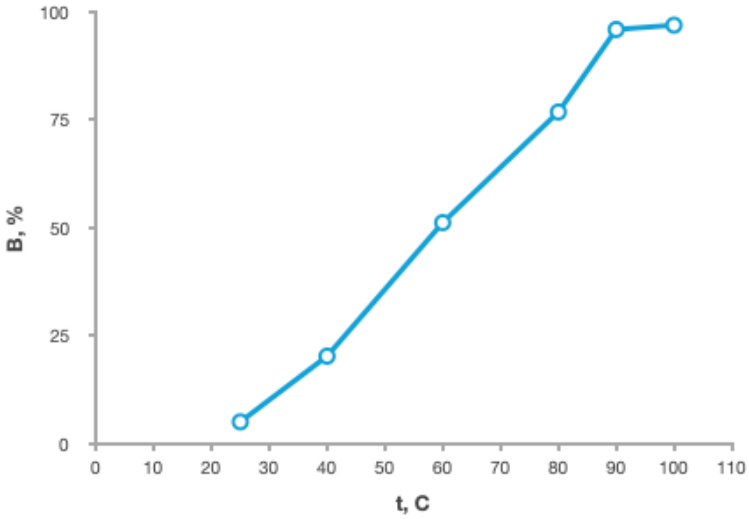


Figure 28 – Temperature dependence of ^{177}Lu-DOTAELA output (± 1.3, p< 0.05) [111]

As can be seen from figure 27, with an increase in temperature from 25 to 100 °C, the B value increases and reaches a maximum at 90-100 °C, this temperature range is optimal.

The optimal radio-labeling parameters were determined: synthesis pH ^{177}Lu-DOTAELA 4.5, temperature 90-100 °C, complexation time 40 minutes. As a result of research, a block scheme for obtaining the ^{177}Lu-DOTAELA complex was developed. According to this scheme, the radio chemical yield is ≥ 95%[111].

3.2.3 Study of the purification and stability of ^{177}Lu-DOTAELA complex

The following reagents were used in the work: distilled water, lutetium-177 chloride, citrate buffer solution with pH-5.0, acetate buffer solution with pH-4.5, acetone, acetonitrile, ethanol. Stock solutions were prepared by dissolving an exact portion of the reagent in distilled water. Solutions of a lower concentration were prepared by diluting the stock solutions. The radiochemical purity of the labelled complex was determined on a VCN-101 radio-chromatogram scanner (VEENSTRA, Germany). Cartridges are pre-conditioned.

Radiolabeling was carried out with the lutetium-177 isotope obtained by irradiating 200 μg of lutetium in the form of chloride with a thermal neutron flux of 2 * 10^{14} n/cm^2* s for 254 hours. After irradiation, the target was held for 6 hours and then transported to a hot chamber to open the ampoules, followed by the dissolution of the target in 2 mL of a 0.01 M hydrochloric acid solution.

For DOTAELA radiolabeling, 100 μL of a DOTAELA with concentration of 1 mg/ml was taken into a 10 mL vial, then 125 μL of an acetate buffer solution with pH 4.5 was added, then 50 μL of lutetium-177 chloride was added and the volume was adjusted

to 2 mL with distilled water. The final mixture was placed in a glycerin bath at 90-95 °C for 60 minutes. At the end of time, the vial was removed and cooled, then the solution was passed through a sorbent cartridge. Chromatography samples were taken in stages before and after passing the solution through the cartridge. FN1 type chromatographic paper was used as the stationary phase. 5 μL of the test solution was applied to the start line at a distance of 2 cm from the beginning of the chromatographic strip, after applying the spot, it was dried. Then the strips were placed in a sealed chamber, and one end of it was immersed in the mobile phase [133]. As the mobile phase was used: 0.1 M buffer solution of sodium citrate, pH 5.0.

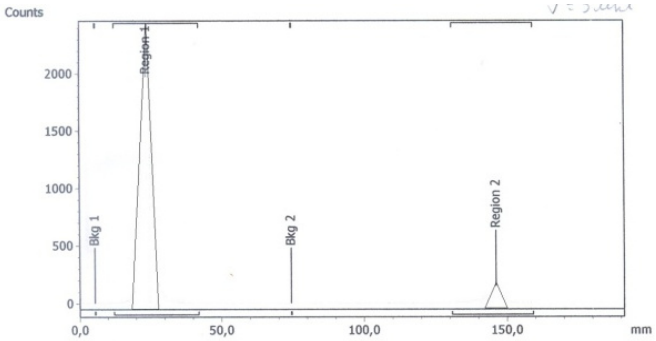
Experiments 1-6 were performed by determination of radiochemical yield ^{177}Lu-DOTAELA with/without purification (table 11), consisting of three stages: conditioning, washing and elution. Ethanol/water and acetonitrile/water 50:50 (V:V), respectively, were used as eluents.

The radiochemical yield of ^{177}Lu-DOTAELA was measured using a radio chromatogram scanner for 48 hours after synthesis. In figure 29A shows a typical ^{177}Lu-DOTAELA radio chromatogram, which was prepared as described in the experimental part, and a sample was taken after synthesis. Figure 29B shows the ^{177}Lu-DOTAELA radio chromatogram in the absence of components that reduce the effect of radiolysis, after 48 hours.

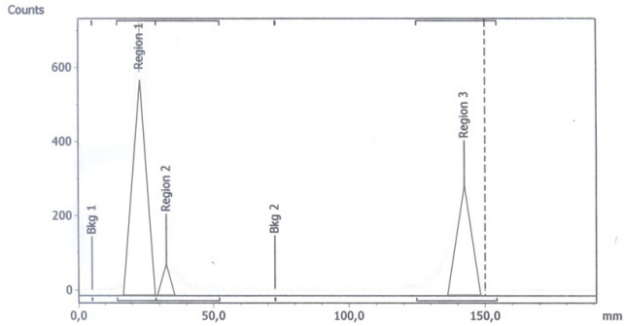
Table 11 - Design of an experiment to study purification

Experiment	1	2	3	4	5	6
Sorbent	-	SepPac C18	SCX	Dowex 50W-X-12	Purolite WCA 100	КУ-2
Sorbent pretreatment	-	+	+	+	+	+
Flushing	-	+	+	+	+	+
Elution	-	+	+	+	+	+
The final volume, ml	1	1	1	1	1	1
Total activity, GBq	3.7	3.7	3.7	3.7	3.7	3.7
3.7GBq/ml	+	+	+	+	+	+

In figure 29, the main ^{177}Lu-DOTAELA peak detected by a scintillation detector (NaI) is located on the start line, and the peak corresponding to free $^{177}Lu^{3+}$ moves along the chromatogram along with the solvent front.



A



B

Figure 29 - Chromatograms: A- ^{177}Lu-DOTAELA after 1 h and B- ^{177}Lu-DOTAELA after 48 hours [134]

^{177}Lu-DOTAELA without purification of C18 and the use of compounds that reduce the effect of radiolysis after radiolabeling loses stability after 24 after the formation of the complex.

To investigate the effects of purification, we performed radiosynthesis, then purged using cartridges filled with C18 and various cation exchangers. When performing synthesis with a radioactive label, the initial radiochemicalyield was from 90 to 93%, however, after purification using C18 and cation exchangers, purification led to the complete elimination of the labelled ^{177}Lu-DOTAELA complex and free $^{177}Lu^{3+}$.

The purification process consisted of three parts: adsorption, washing and elution. According to the data obtained in experiments 2-6 on a C18 cartridge and filled with various cation-exchangers, the $^{177}Lu^{3+}$ cation is retained, as well as the ^{177}Lu-DOTAELA complex is retained. In the case of the C18 cartridge, the results obtained are consistent with theory, however, in the case of cation exchangers, not retention occurs, but the destruction of the complex and the use of cation exchangers reduces to the search for weaker cation-exchangers. In this regard, the washing stage in the case of cation-exchangers of this type was not carried out.

Distilled water and an acetate buffer solution with a pH of 5.0 were used to rinse the C18 cartridge. In both cases, as a result of washing, free $^{177}Lu^{3+}$ is removed from the cartridge, and the complex remains. Sequential elution of the complex from the C18 cartridge after washing with ethanol and acetonitrile did not give satisfactory results. The volume of eluents ranged from 5 ml to 100 ml, with a complex yield of 10-15%. Based on these results, it can be concluded that the standard implementation of C18 purification, which is usually effective for eliminating unincorporated $^{177}Lu^{3+}$ ions, requires the use of compounds that reduce the effect of radiolysis, for example, ascorbic acid, to maintain ^{177}Lu-DOTAELA radiochemicalyield, as well as search for other eluents. For example, the use of trifluoroacetic acid to increase the polarity of the solvent.

Figure 30 shows the stability of the ^{177}Lu-DOTAELA complex without purification for 48 hours and without the use of compounds that reduce the radiolysis effect after the radiolabeling process.

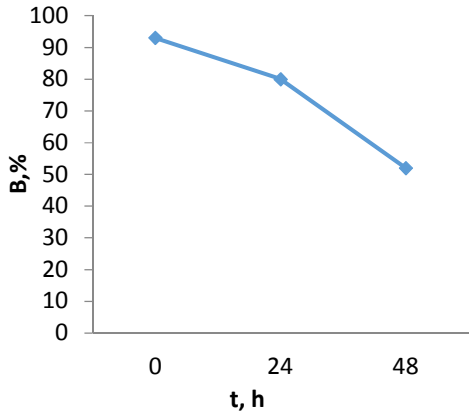


Figure 30 - Complex stability of ^{177}Lu-DOTAELA (±0,9, $p<0.05$) [134]

As can be seen from the figure, the complex has sufficient radiochemical purity within 24 hours after the formation of the complex.

3.2.4 Development of the composition of the reagents for obtaining isotonic solution ^{177}Lu-DOTAELA

The introduction of injection solutions, the osmotic pressure of which differs from the osmotic pressure of the blood plasma, causes sharp pain, the sensation of which is stronger, the greater the osmotic difference. The sharp pain felt when using injectable solutions is eliminated by injecting excipients to isotonize the solution.

The osmotic pressure of a multicomponent solution according to Dalton's law [3] consists of the partial osmotic pressures of the individual components (equation 13):

$$P = P_1 + P_2 + P_{3...} \quad \text{и т. д.} \qquad (13)$$

Each of the components accounts for the isotonization of the corresponding volume of solution in milliliters (equations 14, 15).

$$V_{общ} = v_1 + v_2 + v_3, \text{ откуда} v_3 = V_{общ} - (v_1 + v_2) \quad (14)$$

$$V_1 = \frac{1000 m_1 i_1}{0.29 M} \quad (15)$$

In the present work, the starting components were taken in ratio 1: 1 according to the chemical reaction equation 16. To 200 μL of DOTAELA solution, then 125 μL of an acetate buffer solution with pH 4.5 was added, after which lutetium-177 chloride dissolved in 0.01 M hydrochloric acid was added. The total volume is adjusted with water for injection to 2 mL[136].

$$\text{DOTAELA} + {}^{177}Lu^{3+} \rightarrow {}^{177}\text{Lu-DOTAELA} + 3H^{+} \quad (16)$$

The product contains:

- complex ^{177}Lu-DOTAELA – 232.8 μg
- sodium acetate – 0.0079 g

The concentration of the ^{177}Lu-DOTAELA complex and hydrochloric acid are such that they practically do not affect the osmotic pressure. Perform calculations for sodium acetate[136].

$$V(CH_3COONa) = (1000*0.0079*1.86)/(0.29*71) = 0.71 \text{ mL}$$
$$V(NaCl) = V_{общ} - V(CH_3COONa) = 2 - 0.71 = 1.29 \text{ mL}$$

The mass of sodium chloride for isotonizing the solution is calculated [136]:

$$m(NaCl) = (0.29*58.5*1.29)/(1000*1.86) = 0.012 \text{ g}$$

Adding 0.012 g of sodium chloride results in an isotonic solution.

3.2.5 Methods of preparing the dosage form of the radiopharmaceutical ^{177}Lu-DOTAELA

Based on the preliminary development of the optimal composition (table 12) of the ^{177}Lu-DOTAELA preparation and optimal radiolabeling conditions, the following block scheme was used to develop prototype radiopharmaceuticals in order to conduct quality control and study functional suitability:

- Preparation of solutions for the preparation of a mixture of reagents included in the composition and excipients.
- Target irradiation
- Preparation of bulk solution
- Radiolabelling
- Filtration and sterilization
- Quality control

In accordance with the developed technological scheme, 200 μL of a solution of DOTAELA in ethanol were taken into a 10 mL vial with pre-weighed sodium chloride weighing 0.012 g, then 125 μL of an acetate buffer solution with a pH of 4.5 was added, then lutetium-177 chloride was added with an activity of ≈ 7,4 GBq dissolved in 0.01 M hydrochloric acid was adjusted to 2 mL with distilled water. The final mixture was placed in a glycerin bath at 90-95 °C for 40 minutes. At the end of time, the vial was removed and cooled. At the end of the process, samples of preparations with a volume of 5 μL were taken from vials for chromatography and radiometric measurements.

Table 12 - Composition of the radiopharmaceutical «^{177}Lu-DOTAELA» in a bottle

Materials	The bottle of the finished product contains
Lutetium - 177	6.5 – 8.1 GBq
DOTAELA	200 μg
Hydrochloric acid	0.001M
Sodium Chloride	12 mg
Sodium Acetate	7.9 mg
Water for injections	till 1.8 mL

The resulting preparation has a radiochemical purity of 93-96%, which allow sit to be used for biomedical tests.

3.2.6 Block scheme for obtaining the radiopharmaceutical ^{177}Lu-DOTAELA

As a result of studies to determine the optimal synthesis parameters of the ^{177}Lu-DOTAELA complex, a block scheme has been developed (figure 31).

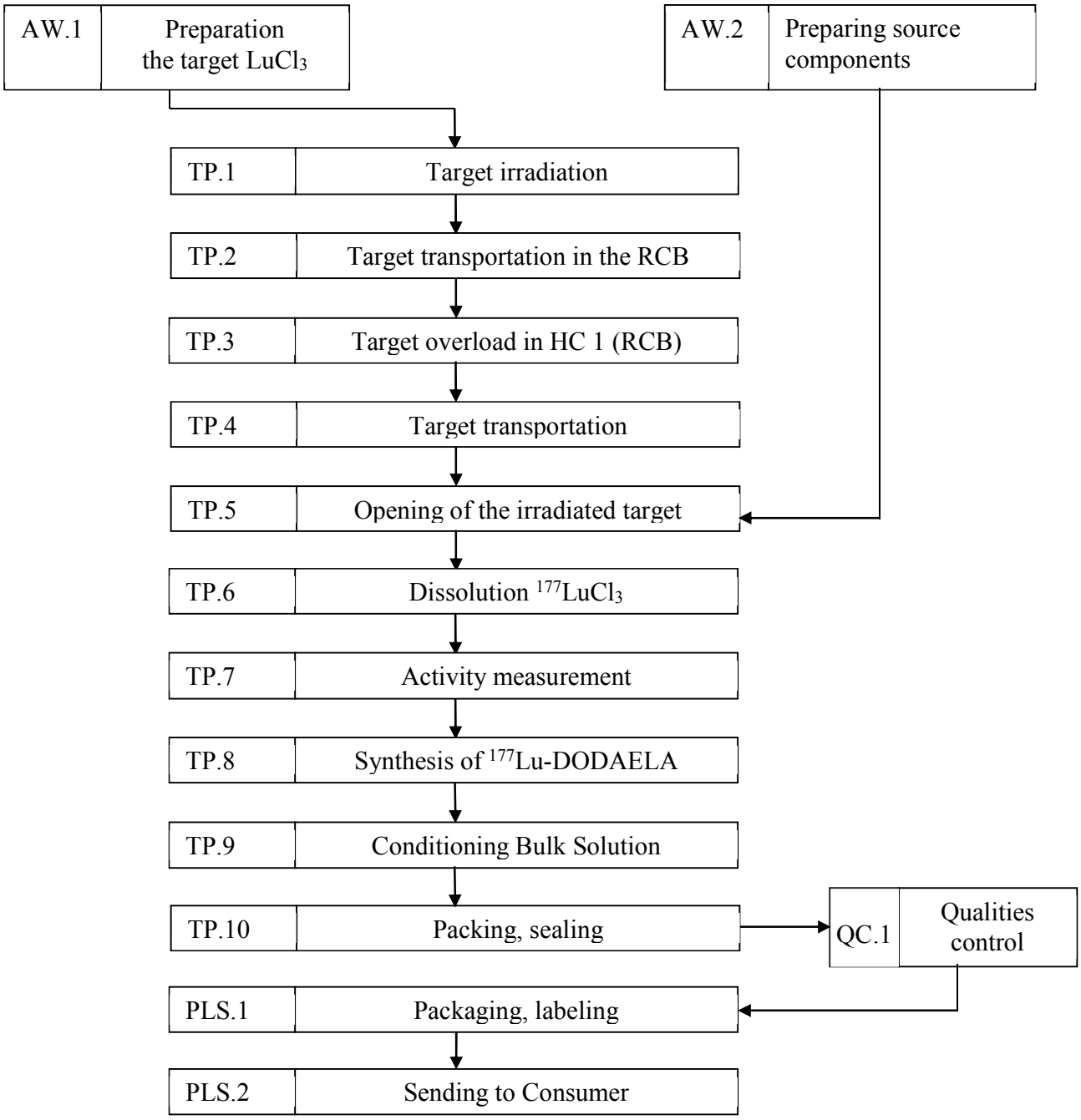


Figure 31 - The block scheme of obtaining ^{177}Lu-DOTAELA

Based on the developed technological scheme, a control synthesis of DOTAELA radio-labeling was carried out. 100 μL of DOTAELA solution with a solution concentration of 1 mg/mL was taken into a 10 mL vial, then 125 μL of acetate buffer solution with pH 4.5 was added, then 50 μL of lutetium-177 chloride was added and the volume was adjusted to 2 mL with distilled water. The final mixture was placed in a glycerin bath at 90-95 °C for 40 minutes.

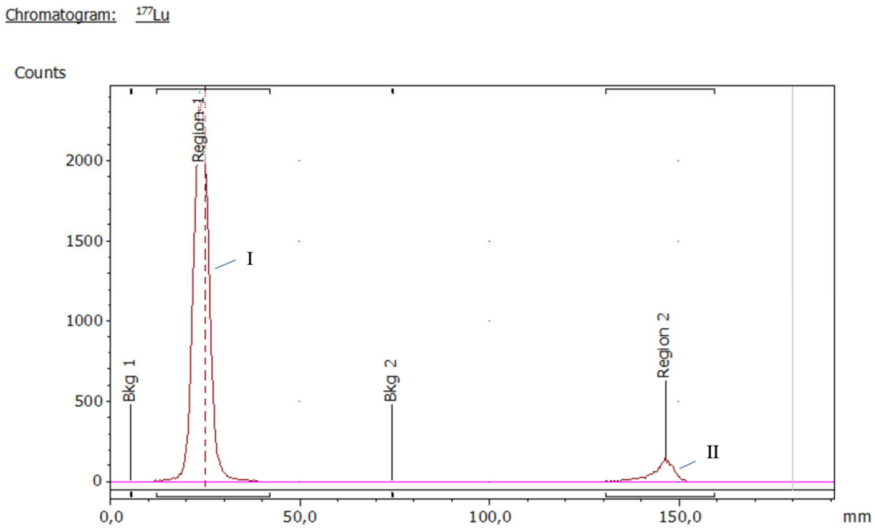


Figure 32 - Radiochromatogram of ^{177}Lu-DOTAELA. I - ^{177}Lu-DOTAELA, II - $^{177}Lu^{3+}$[48]

When performing synthesis after determining the optimal synthesis parameters, the RCP was 96% (figure 32).

3.3 Selection and verification of quality control methods and preparation of draft specifications for the production of pilot batches.

The requirements for the quality control of radiopharmaceuticals can be found in Volume 3 of the State Pharmacopoeia of the Republic of Kazakhstan (SP RK) is the main regulatory document in our country, in the absence of specific monographs and articles in the national pharmacopoeia, it is allowed to use and refer to pharmacopoeias of other countries (USA, Japan, Great Britain and Europe). Basic requirements for radiopharmaceuticals: radionuclide identification; radiochemical purity; chemical purity; pH, residual solvents; sterility and level of bacterial endotoxins; checking the integrity of the filter membrane. At the same time, all determined quality indicators of the drug and methods for their control are given in the Specification for the RP.

Almost all of the listed indicators are determined by standard methods described in the methodological part of the work or given in the State Pharmacopoeia of the Republic of Kazakhstan.

The active compound of the RP being developed for the treatment of triple-negative breast cancer is a complex of the radioactive isotope of lutetium ^{177}Lu and the non-peptide antagonist gonadotropin-releasing hormone (GnRH) elagolix (^{177}Lu-DOTAELA). Receptors for triple-negative breast cancer show expression of gonadotropin-releasing hormone (GnRH) in more than 50% of cases [2]. Elagolix, marketed under the brand name Orilissa, which is used to treat pain associated with endometriosis in women. It is also

being developed to treat uterine fibroids and heavy menstrual bleeding in women. The analytical methods used to determine the components of the drug require careful verification, taking into account the possible mutual influence of these components on the result of their determination.

The purpose of this stage is to develop a quality control methodology.

3.3.1 Identification

The determination of ^{177}Lu is carried out by gamma spectrometry by measuring the energy of ^{177}Lu gamma radiation.

Test solution (a). Test drug.

Test solution (b). 10 μL of the test solution (a) is diluted in 10 mL of water P, mixed, after which 10 μL of the test solution (b) is applied to filter paper with a size of 20x20 mm and the paper is sealed with adhesive tape.

The measurements are carried out on a gamma spectrometer with a detector of high purity germanium.

The ^{177}Lu gamma spectrum of the test solution should have a characteristic line with the energy of 0.208 MeV.

The determination of ^{177}Lu-DOTAELA is carried out by liquid chromatography (SP RK I, v.1, 2.2.29) when tested for radiochemical purity.

The relative retention time of ^{177}Lu-DOTAELA should differ by no more than 3% from the retention time of the non-radioactive Lu-DOTAELA complex.

3.3.2 pH – test

The determination of pH is carried out potentiometrically (SP RK I, t. 1, 2.2.3). The pH of the test drug should be in the range of 4.5 to 8.5.

3.3.3 Transparency - test

The determination is carried out by viewing with the naked eye along the vertical axis of the vial with the test drug and the vial of water P on a black background illuminated by a twenty-watt fluorescent lamp. The distance from the eye to the viewed object should be twenty-five to thirty centimeters, and the angle of the optical viewing axis to the direction of light is about ninety degrees. The line of sight should be directed slightly downward with the vertical position of the head (SP RK I, t. 1, 2.2.1).

The surface of the viewed bottles should be clean and dry from the outside.

The drug should be transparent compared to water R.

3.3.4 Determination of chromaticity

The determination is carried out by viewing with the naked eye along the horizontal axis of the vial with the drug and the vial of water P on a white background illuminated by a twenty-watt fluorescent lamp. The distance from the eye to the viewed object should be twenty-five to thirty centimetres, and the angle of the optical viewing axis to the

direction of light is about ninety degrees. The line of sight should be directed slightly downward with the vertical position of the head (SP RK I, t. 1, 2.2.2).

The surface of the viewed bottles should be clean and dry from the outside.

The drug should be colorless compared to water P or the color of the drug should not be more intense than the color of the comparison solution Y_7.

3.3.5 Test procedure for mechanical inclusions

The determination is carried out in accordance with the requirements of SP RK I, t. 1, 2.9.20 (visible particles) by viewing the solution with the naked eye on black and white backgrounds illuminated by a twenty-watt fluorescent lamp. The distance from the eye to the viewed object should be twenty-five to thirty centimetres, and the angle of the optical viewing axis to the direction of light is about ninety degrees. The line of sight should be directed slightly downward with the vertical position of the head.

The surface of the viewed bottles should be clean and dry from the outside.

Mechanical inclusions should be absent.

3.3.6 Determination of sodium chloride

Quantitative determination is carried out by direct titration.

10 mL of water P and 0.1 mL of a 5% potassium chromate P solution are added to 0.2 mL of the test drug, stirred and titrated with a 0.02 M silver solution of nitrate P until orange-yellow color.

Before the tests set the titer of 0.02 M solution of silver nitrate R.

1 mL of a 0.02 M solution of silver nitrate P corresponds to 2.92 mg of sodium chloride.

The sodium chloride content in 1 ml of the drug should be in the range of 8 to 10 mg.

3.3.7 Testing bacterial endotoxins

Tests are carried out by the turbidimetric kinetic method for the limiting content of endotoxins (SP RK I, v. 1, 2.6.14).

25 μL of the test drug is introduced into each of the 4 channels of the cartridge for the test system for determining bacterial endotoxins.

The four cartridge channels contain a LAL reagent and a chromogenic substrate. In two of the four channels, an additional control standard of endotoxin with a concentration of 5.0 to 0.05 IU/mL was added.

Measurements are carried out on an automatic test system with a spectrophotometer.

The content of bacterial endotoxins should not exceed 175/V ME/mL, where V is the maximum recommended dose in mL, 2 mL.

3.3.8 Sterility test procedure

Tests are carried out by direct seeding on nutrient media (SP RK I, t. 2, 2.6.1)

The drug must be sterile.

3.3.9 Determination of radionuclide impurities

The determination of gamma-emitting radionuclide impurities is carried out by gamma spectrometry (SP RK I, t. 3, 0125).

10 μL of the test solution (b) is applied to 20x20 mm filter paper and the paper is sealed with adhesive tape.

The measurement is carried out on a gamma spectrometer with a detector of high purity germanium.

The content of gamma-emitting radionuclide impurities should not exceed a total of 0.1% of total radioactivity.

3.3.10 Determination of radiochemical purity (HPLC)

The ^{177}Lu-DOTAELA impurity test is carried out by liquid chromatography (SP RK I, v.1, 2.2.29) on a chromatograph with a detector suitable for measurement in the required concentration range on a chromatographic column suitable for separation.

3.3.11 Determination of radiochemical purity (PC)

About 5 μL of test solution (a) is applied to the start line of the paper chromatographic strip. The strip is placed in a chamber with 1 M buffer solution of sodium citrate, pH 5.0. When the solvent front has passed a distance of more than 12 cm from the start line, the plate is removed and dried in air for 15 minutes.

The distribution of radioactivity in the chromatogram of the test solution is carried out using a suitable detector.

The retention factor ^{177}Lu-DOTAELA should be around 0; the retention factor of ^{177}Lu is about 0.90 to 1.00.

At least 95% of the total radioactivity of ^{177}Lu should be in ^{177}Lu-DOTAELA.

3.3.12 Determination of inactive impurities

SP RK I, t.1, 2.2.22

Calibration solutions are prepared:

450 mg of NaCl are added into four volumetric flasks with a capacity of 100 mL, about 10 mL of bidistilled water are added to dissolve NaCl and 1 mL of Sc solution with a concentration of 0.1 mg/mL is added. Then, an automatic pipette and a 1 mL pipette are added to the flasks, respectively, 0 (blank solution); 0.05; 0.1 and 0.5 mL of each of the eight intermediate RCM solutions with a concentration of 0.1 mg/mL, bring the volume of the solution to the mark with double-distilled water and mix. 1 mL of calibration

solutions contains 0, respectively (blank solution); 0.05; 0.1 and 0.5 μg of the following elements: Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Zn.

Sample preparation for analysis:

1.6 mL bidistilled water and 0.4 mL Sc solution with a concentration of 0.01 mg/mL is poured into a 10 mL bottle equipped with a label.

Inactive impurities should not be detected in quantities that exceed their detection limits (table 13).

Table 13 – maximum permissible concentration, mg/mL

Element	Al	Cr	Ni	Cu	Zn	Pb	As	Sn	Fe	Mn
Normative Index	2.0	0.1	0.1	0.05	2.5	0.2	1.0	0.1	0.25	0.03

3.3.13 Determin at ion of residual solvents

The test for the presence of ethanol is carried out by gas chromatography (SP RK I, v. 1, 2.2.28).

Test solution (a). Test drug.

Chromatograph 1 μL of the test solution (a) and 1 μL of the comparison solution (f).

In the chromatogram of the test solution (a), the peak area of the impurity shall not exceed the area of the corresponding peak in the chromatogram of the comparison solution (f).

Comparison Solution (f). Add 50 mg of ethanol R to the vial, add water R to the volume of solution V, where V is the maximum recommended dose in milliliters - 2 mL.

The ethanol content should be no more than 50 mg/V (25.0 mg/mL), where V is the maximum recommended dose in milliliters, 2 mL.

3.3.14 Determination of volumetric activity

Volumetric activity ^{177}Lu in the preparation is determined by gamma spectrometry.

Test solution (d). 10 μL of the test solution (a) is diluted in 10 mL of water P and mixed, after which 10 μL of the test solution (d) is applied to 20x20 mm filter paper and the paper is sealed with adhesive tape.

The measurements are carried out on a gamma spectrometer with a detector of high purity germanium.

Volumetric activity ^{177}Lu should be at least 3.7 MBq/mL.

3.3.15 Draft specification on the radiopharmaceutical «^{177}Lu-DOTAELA»

Based on the studies carried out in the work on the development of the composition of the radiopharmaceutical «^{177}Lu-DOTAELA», as well as the above methods for

analyzing its quality, a draft specification was developed (table 14) for the production of experimental batches of radiopharmaceuticals shown in the table.

Table 14 – Draft specification for the radiopharmaceutical «^{177}Lu-DOTAELA», solution for intravenous administration [137]

Test	Method	Requirements
1	2	3
Identification ^{177}Lu-DOTAELA	HPLC (SP RK I, т.1, 2.2.29)	The relative retention time of ^{177}Lu-DOTAELA should differ by no more than 3% from the retention time of the non-radioactive complex Lu-DOTAELA.
Identification^{177}Lu	Gamma-spectrometry	The ^{177}Lu gamma spectrum of the test solution should have characteristic lines with energies of 0.113 and 0.208 MeV.
pH	Potentiometry (SP RK I, т. 1, 2.2.3)	from 4.5 to 8.5
Transparency	SP RK I, т. 1, 2.2.1	Transparent compared to water P
Chromaticity	SP RK I, т. 1, 2.2.2	The color of the drug should not be more intense than the color of the solution of comparison Y7
Mechanical inclusions	SP RK I, т. 1, *2.9.20*	Mechanical inclusions must be absent
Sodium chloride	Direct titration	from 8 to 10 mg
Radionuclide impurities	Gamma-spectrometry	The content of gamma-emitting radionuclide impurities should not exceed a total of 0.1% of total radioactivity.
Radiochemical purity	Chromatography on paper (SP RK I, V. 1, *2.9.26)*	Not less than 95%
Residual Solvents	Gas chromatography (SP RK I, т. 1, 2.2.28)	The ethanol content should be no more than 50 mg/V
Sterility	Direct culture inoculation (SP RK I, V. 2, 2.6.1)	Sterile

Table 14 continued

1	2	3
Bacterial end otoxins	Turbidimetric kinetic method (SP RK I, V. 1, 2.6.14)	Should not exceed 175/V ME/mL
Inactive impurities	Inductively coupled plasma atomic emission spectrometry SP RK I, V.1, 2.2.22	Inactive impurities should not be detected in quantities that exceed their detection limits (table 13)
Packaging		For packaging use the transport packaging set UKTIA-10 GOST 16327-88. The packing kit is sealed with lead seal.
Marking		Labeling is carried out in accordance with the «Rules for the Labeling of Medicines, Order of the Minister of Health and Social Development of the Republic of Kazakhstan» dated 04.16.2015, No. 22
Transportation		Transportation is carried out in accordance with the «Rules for the transport of radioactive materials and radioactive waste», order of the Minister of Energy of the Republic of Kazakhstan dated 02.22.2016, No. 75
Shel flife		No more than 24 hours from the time of preparation
Precautions		Must comply with the «Sanitary and epidemiological requirements for radiation safety»

3.3.16 Production of pilot batches

Based on the block scheme for the synthesis of the radiopharmaceutical "^{177}Lu-DOTAELA", three series of the drug were produced under the conditions of compliance with the requirements of good manufacturing practice [1, 2].

The production was carried out in several stages based on the developed documented procedure "Organization of production of radiopharmaceuticals in the RIPC INP ME RK": irradiation of the prepared target, preparation of equipment and raw

materials, implementation of the production process in a hot chamber, product quality control.

Before the start of production of each batch of the preparation, hot chambers, a loading gateway, a dose calibrator and a Thimotheo-LT filling device were prepared. All preparatory procedures were performed before the irradiated target was delivered to the hot chamber.

Preparation for the operation of the hot chambers and the loading gateway included checking the vacuum in the chambers and the gateway, connecting to power, connecting dosimetric equipment.

The preparation of the Capintec CRC-25R dosing calibrator consisted in calibrating the instrument with a standard Cs-137 source and measuring the background.

The preparation of the Thimotheo-LT filling device consisted of calibrating the scales built into the device using a standard with a known mass.

Figure 33 shows the preparation process for the synthesis of the radiopharmaceutical «^{177}Lu-DOTAELA».

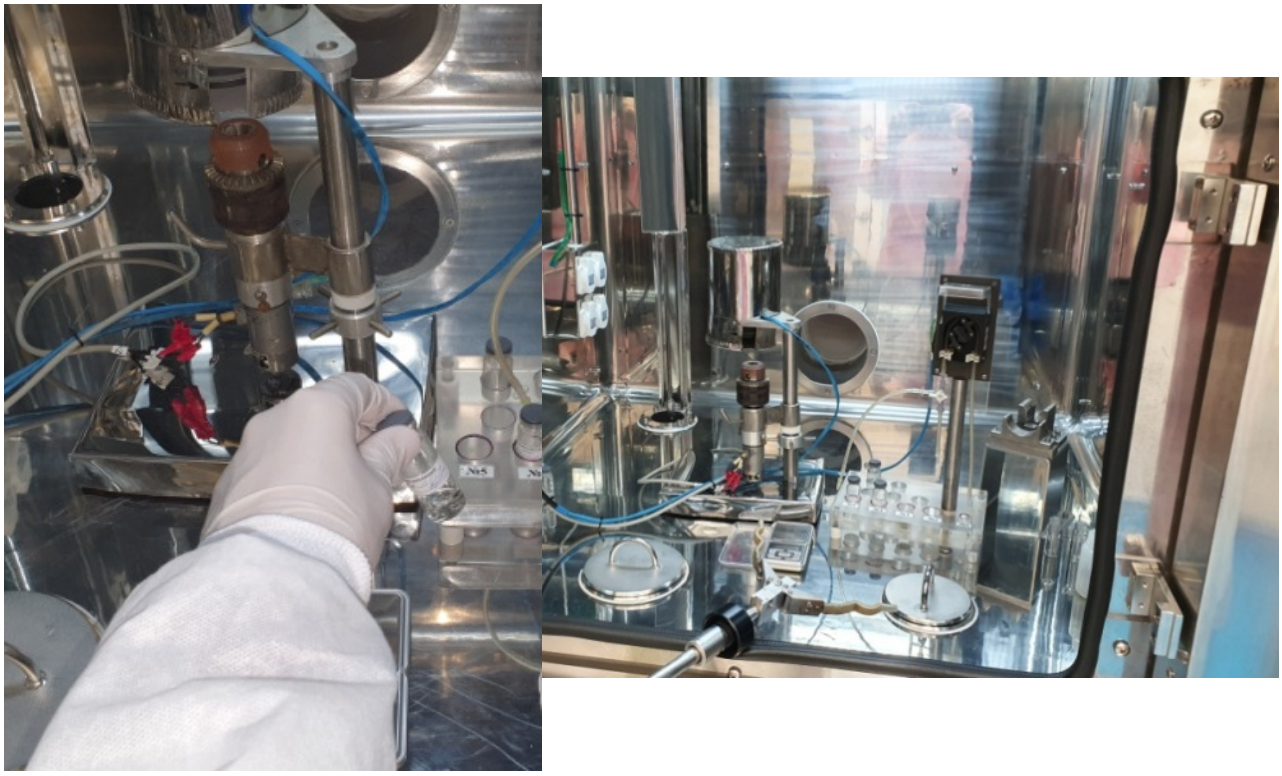


Figure 33 - Preparations for the synthesis

The parameters of irradiation and synthesis of the experimental series «^{177}Lu-DOTAELA» are presented in table 15.

Table 15 - Parameters of irradiation and synthesis of experimental series «^{177}Lu-DOTAELA»

Irradiation time, min	The activity of ^{177}Lu after dissolution, GBq	Time synthesis	Activity ^{177}Lu-DOTAELA at the end of the synthesis, GBq
252	88.4	40 min	7.6
408	152.30	40 min	7.4
240	53.4	40 min	7.5

After completion of the synthesis, the obtained preparation was dosed into vials using a filling device, one of which was used for quality control. Vials with the packaged preparation were sealed with rubber stoppers and crimped with aluminum caps, after which the activity of ^{177}Lu in each bottle was measured. In series, the vials were placed in protective containers and unloaded from the hot chamber. When unloading, a label was glued onto the container. Figure 34 shows an example label.

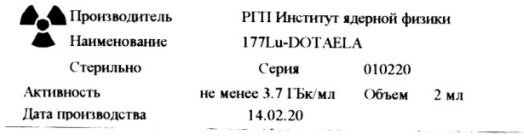


Figure 34 - An example of a label on a bottle with the drug «^{177}Lu-DOTAELA» and shipping container

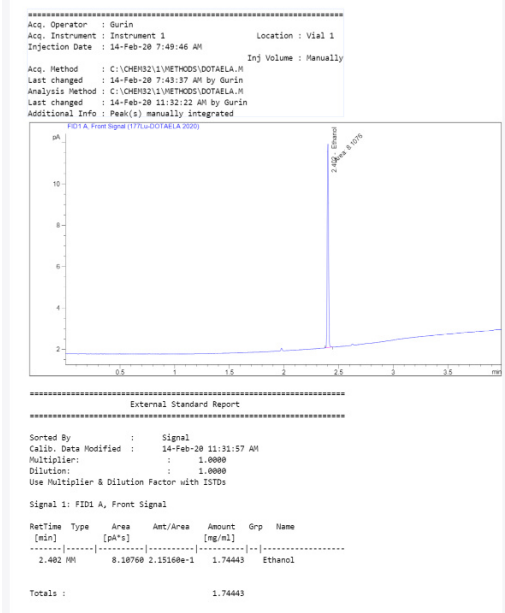
Figure 35 shows the process of unloading the container with the drug from the hot chamber, and figure 36 shows the process of transferring the drug to the laboratory for quality control.

Figure 35 - Unloading the drug container from the hot chamber

Figure 36 - Transfer of the container with the drug for quality control through a ventilated gateway

Quality control of the experimental series included the determination of volumetric activity, radionuclide and radiochemical purity, residual solvent content, and pH.

Figures 37 and 38 show graphical images of the results of tests to determine the content of residual solvents by gas chromatography and radiochemical purity by paper chromatography.

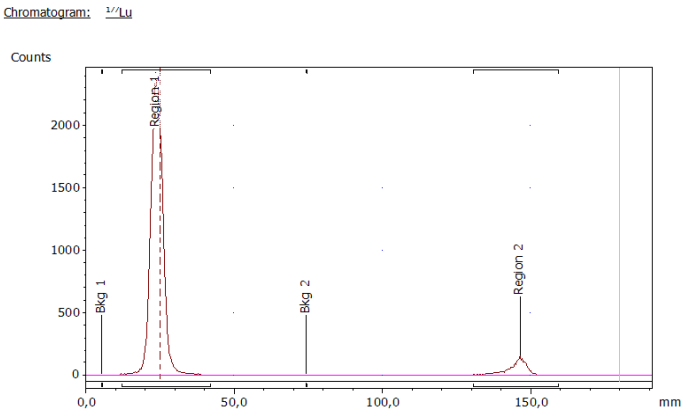


```
Acq. Operator   : Gurin
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 14-Feb-20 7:49:46 AM
                                                  Inj Volume : Manually
Acq. Method     : C:\CHEM32\1\METHODS\DOTAELA.M
Last changed    : 14-Feb-20 7:43:37 AM by Gurin
Analysis Method : C:\CHEM32\1\METHODS\DOTAELA.M
Last changed    : 14-Feb-20 11:32:22 AM by Gurin
Additional Info : Peak(s) manually integrated
```



```
                    External Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      14-Feb-20 11:31:57 AM
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [pA*s]                 [mg/ml]
-------|------|----------|----------|----------|--|----------------
  2.402 MM       8.10760 2.15160e-1    1.74443    Ethanol


Totals :                               1.74443
```

Figure 37 - Determination of residual solvent content by gas chromatography in the radiopharmaceutical «^{177}Lu-DOTAELA»



Regions: ^{177}Lu

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Bkg 1	5,4	5,6	-0,127			
Region 1	12,2	42,0	-0,009	56780	93,01	96,32
Bkg 2	74,4	74,6	0,319			
Region 2	130,8	159,2	0,782	4264	6,99	2,93
2 Peaks				61044	100,00	99,25

Figure 38 - Determination of radiochemical purity of the radiopharmaceutical «^{177}Lu-DOTAELA» by thin layer chromatography

The results of the quality control of the RP «^{177}Lu-DOTAELA» are presented in table 16.

Table 16 - Test results of experimental batches of radiopharmaceutical«^{177}Lu-DOTAELA»

No. series	pH	Volumetric activity, GBq/mL	RNP, %	RCP,%		The content of residual solvents, mg/mL (Ethanol)	Microbiological purity
				^{177}Lu-DOTAELA	$^{177}Lu^{3+}$		
Requirement	4.5-8.5	3.4-3.8	99.8±0.005 %	≥95.0%	≤ 5%	≤6.25	Sterile
010220	7.3	3.8	99.9	96.3	3.6	1.7	Sterile
020220	7.6	3.7	99.9	95.5	4.2	0.9	Sterile
030320	7.4	3.8	99.9	96.7	2.8	1.4	Sterile

3.4 Chapter Summary

1. The effect of irradiation parameters of ^{176}Lu was studied to produce high specific activity of ^{177}Lu «direct» in the WWR-K reactor.

2. The mobile phase for chromatographic studies of DOTAELA labelled with isotope labelled with lutetium -177 was selected.

3. The optimal parameters of DOTAELA radio-labeling with the lutetium-177 isotope were determined. Study of the purification process of the ^{177}Lu-DOTAELA complex. Study of the stability of the ^{177}Lu-DOTAELA complex. Development of the composition of the reagents for obtaining the isotonic solution ^{177}Lu-DOTAELA. The block scheme is developed.

4. Methods of analytical quality control of the radiopharmaceutical «^{177}Lu-DOTAELA» according to «Good Manufacturing Practice (GMP)» have been developed. The methods of qualitative and quantitative determination of the main components in the composition of the developed RP were tested.

5. A draft Specification has been proposed for producing pilot batches of a new domestic radiopharmaceutical as the main section in the preparation of a pharmacopeia article for the manufacture of a medicinal product.

6. Three pilot batches of the drug were manufactured.

CONCLUSION

The aggressiveness of the course of TNBC and the difficulty of conducting therapy among patients, combined with the impact on the quality of life and health, just determines the need for development in the search for new selective and specific drugs and the ability to use invasive measures only as a last resort.

For a long time, it was believed that TNBC cells do not have receptors on their surface. Further study showed that cells show expression for GnRH in 50% of cases.

Based on the foregoing, it can be concluded that in the cells of a breast tumor, the presence of GnRH receptors increases the likelihood of accumulation of GnRH analogues, which, in turn, creates prospects for the development of radiopharmaceuticals for the diagnosis and treatment of TNBC and makes research on the synthesis of new highly informative drugs.

Of the wide variety of commercially available GnRH analogues, nonpeptide antagonists are of particular concern. Of particular interest is because these representatives have a lower molecular weight compared to GnRH agonists and peptide antagonists, which in turn affects the clearance of a compound in the body.

Our interest was attracted by the GnRH antagonist Elagolix approved by the FDA Quality Management. Because it is not possible to directly label elagolix with the lutetium-177 isotope, at the University of Oslo, the elagolix was connected to the DOTA chelating agent via an ethylene diamine bridge (DOTAELA).

The specific biomolecule used, carrying with it a radioactive isotope, not only inhibits cell growth, but also undergoes β-irradiation, which allows to achieve a double therapeutic effect. Unfortunately, the development process takes many years. The synthesis of a molecule with selectivity, the development of procedure, research in vivo and in vitro requires time and cost. In our case, the synthesis of the active compound is synthesized at the University of Oslo, at the Institute of Nuclear Physics in Almaty, this molecule is labelled and the quality of the final preparation is controlled.

The generally recognized trend in the development of radioisotope therapy over the past 2 decades is the use of the ^{177}Lu isotope, its chemical and nuclear-physical characteristics ensure the demand for this isotope in the world in the future.

Currently, there are known works in which attempts are being made to create drugs of various compositions based on ^{177}Lu-labelled derivatives of GnRH analogues. However, almost all of the drugs under development are aimed at the $HER2^{+}$ receptors of the technique, while TNRM cells are characterized by a lack of expression ($HER2^{-}$).

To solve this problem in the dissertation for the first time, a reagent was developed to create a drug based on DOTAELA with a sufficient shelf life and, as a consequence, the possibility of its transportation and delivery to other clinics. To do this, it was necessary to solve the following problems: to conduct an experimental verification of existing methods for labeling with lutetium-177. To study the parameters of irradiation of ^{176}Lu and the production of high specific activity of ^{177}Lu «direct» way in the WWR-K

reactor. Select mobile phase systems for radiochromatographic studies of a radiopharmaceutical. To develop a qualitative and quantitative composition of reagents and conditions for the synthesis of ^{177}Lu-DOTAELA with high radiochemical purity of the target labelled product. To develop a procedure for manufacturing a set of reagents, including research on the influence of excipients on the production process and the quality of the developed product. To select and test the methods of quality control of the synthesized preparation «^{177}Lu-DOTAELA» and create a draft Specification for the production of its pilot batches.

The results of the research of the thesis allow formulating the following conclusions:

1. This study showed that 819 GBq / mg Lu-177 with specific activity as a result of a direct nuclear reaction can be obtained by irradiation in a WWR-K reactor with a thermal neutron flux of $1.2 \cdot 10^{14}$ $cm^{-2} \cdot s^{-1}$. Lu-177m is formed during irradiation and is a parasitic radioisotope during this irradiation time, and its activity is about 2.5% of that of Lu-177. Thus, the possibility of serial production of Lu-177 at the WWR-K reactor was demonstrated. One of the fundamental aspects is the specific activity of lutetium-177, which is a measure for evaluating its use in targeted radionuclide therapy.

The result of this work is recommendations for increasing the specific activity of lutetium-177:

- the use of a nuclear reaction where the target is ytterbium to produce lutetium-177 (indirect nuclear reaction). In the reference [97, 105] this method makes it possible to obtain a high specific activity of lutetium-177; a distinctive feature is the radiochemical separation of the target naclide from the target material;

-to analyze the possibility of creating a neutron trap to increase the neutron flux and the fraction of thermal neutrons in the integral neutron flux.

2. As shown by experiments, when applying the method described in the work [35], the yield of the labelled target product before filtration did not exceed 48% and in the filtrate not more than 3%. Most of the activity of ^{177}Lu remained unreacted or adsorbed on the C-18 column. At the same time, an additional peak corresponding to the radiolysis product was detected on chromatograms. Since there is a partial decomposition of the formed complex, such a drug cannot be considered safe and effective.

Experiments were carried out to select the composition of the mobile phases for radiochromatographic studies of lutetium-177-labelled DOTAELA and synthesis products. The main ^{177}Lu-DOTAELA peak detected by a scintillation detector (NaI) after using an aqueous solution of sodium citrate as the mobile phase is located on the start line ($R_f = 0$), and the peak corresponding to free Lu-177 moves along with the chromatogram along with the solvent front ($R_f = 1$) It follows that ^{177}Lu-DOTAELA does not interfere with the determination of unreacted Lu-177. It was found that citrate buffer solution has the best parameters for studying the behaviour of ^{177}Lu-DOTAELA and obtaining chromatograms of the products of its interaction with ^{177}Lu. The results obtained by HPLC are consistent with those obtained by paper chromatography. However, the capture of

$^{177}Lu^{3+}$ ions in the reverse phase of an HPLC column does not provide the necessary reliability in the analysis of the radiochemical yield; therefore, in this case, it is preferable to use the method of paper chromatography, which gives an idea of the content of radiochemical forms of ^{177}Lu.

The optimal radio-labeling parameters were determined: synthesis pH ^{177}Lu-DOTAELA 4.5, temperature 90-100 ° C, complexation time 40 minutes. As a result of research, a block scheme for obtaining the ^{177}Lu-DOTAELA complex was developed. According to this scheme, the radiochemical yield is ≥ 95%.

The studied purification process consisted of three parts: adsorption, washing and elution. According to the data obtained in experiments on a C18 cartridge and filled with various cation exchangers, the $^{177}Lu^{3+}$ cation is retained, as well as the ^{177}Lu-DOTAELA complex is retained. In the case of the C18 cartridge, the results obtained are consistent with theory, however, in the case of cation exchangers, not retention occurs, but the destruction of the complex and the use of cation exchangers reduces to the search for weaker cation exchangers. Distilled water and an acetate buffer solution with a pH of 5.0 were used to rinse the C18 cartridge. In both cases, as a result of washing, free $^{177}Lu^{3+}$ is removed from the cartridge, and the complex remains. Sequential elution of the complex from the C18 cartridge after washing with ethanol and acetonitrile did not give satisfactory results. The volume of eluents ranged from 5 mL to 100 mL, with a complex yield of 10-15%. Based on these results, it can be concluded that the standard implementation of C18 purification, which is usually effective for eliminating unincorporated $^{177}Lu^{3+}$ ions, requires the use of compounds that reduce the effect of radiolysis, for example, ascorbic acid, to maintain ^{177}Lu-DOTAELA RP, as well as search for other eluents. For example, the use of trifluoroacetic acid to increase the polarity of the solvent.

The stability of the ^{177}Lu-DOTAELA complex without purification for 48 hours and without the use of compounds that reduce the radiolysis effect after the radiolabeling process loses stability 24 after the complex is formed.

A block scheme for the preparation of a preparation based on DOTAELA was developed, which includes the stages of preparation of the components of the mixture, the preparation of reagents in vials for medicines.

3. The preparation procedure of the ^{177}Lu-DOTAELA preparation has been developed and its quality has been analytically controlled. Methods for the qualitative and quantitative determination of its main components have been developed. A draft Specification has been proposed for producing pilot batches of a new radiopharmaceutical for the manufacture of a medicinal product.

Three pilot batches were produced and experienced quality control was carried out. The product meets the requirements and is suitable for *in vivo* and *in vitro* biological studies.

The practical use of radiopharmaceuticals based on labelled with lutetium-177 DOTAELA will allow for the treatment of TNBC, as well as tumor visualization using a gamma camera, which can significantly reduce the cost of the diagnostic procedure. The

use of such a radiopharmaceutical will provide the opportunity to carry out therapy without invasive methods, increase life expectancy and improve the quality of life.

Prospects for further development of the research topic are to study the expression and internalization of TNBC cells at the laboratory of the University of Oslo, Oslo, Norway.

ACKNOWLEDGMENT

The author expresses his gratitude to his colleagues - employees of the Scientific and Technical Center of the RPI of the Institute of Nuclear Physics in Almaty for help and assistance in the work done.

The author also thanks the staff of the University of Oslo Department for providing the active compound DOTAELA.

The author expresses special gratitude to his mentors - supervisors Uralbekov Bolat Muratovich and Patrick Riss for his help, invaluable advice and the scientific experience provided by him in the development of the RP.

For special contribution to the implementation of the project of the Ministry of Education and Science of the Republic of Kazakhstan, I express gratitude to the project managers Ye. T. Chakrova and I.V. Matveyeva.

This work was supported by the Ministry of Education and Science of the Republic of Kazakhstan [No. AP05134384, 2018].

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Appendix A

Data Sheet of elagolix and DOTAELA

Hycultec

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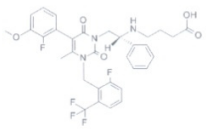
E-Mail: info@hycultec.de
www.hycultec.de

Inhibitors, Agonists, Screening Libraries
www.MedChemExpress.com

Data Sheet

Product Name:	Elagolix
Cat. No.:	HY-14789
CAS No.:	834153-87-6
Molecular Formula:	$C_{32}H_{36}F_5N_3O_5$
Molecular Weight:	631.59
Target:	GNRH Receptor
Pathway:	GPCR/G Protein
Solubility:	10 mM in DMSO



BIOLOGICAL ACTIVITY:

Elagolix is a highly potent, selective, orally-active, short-duration, non-peptide antagonist of the gonadotropin-releasing hormone receptor (GnRHR) (KD = 54 pM).

Target: GnRH

in vitro: Elagolix is a short-acting, nonpeptide, GnRH antagonist, administered orally, that unlike injectable depot GnRH agonists and antagonists, produces a dose-dependent suppression of ovarian estrogen production, that is, from partial suppression at lower doses to full suppression at higher doses. Elagolix is regarded as the frontrunner of a new class of GnRH inhibitors that have been denoted as second-generation, due to their non-peptide nature and oral bioavailability.

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PROFORMA INVOICE

SHIPPER:
Department of Chemistry
Senders Reference: **E17 280034**, Roberto B. Bekker

SHIP TO

Company Name	**INSTITUTE of NUCLEAR PHYSICS (INP)**		
Attention:	**M.Sc. Andrey Gurin** gurenandrey@mail.ru	**AIRWAYBILL No.**	**71 0472 8971**
Address	**1 Ibragimova Street**	Number of pieces:	**1**
Postal Code/ City	**050032 Almaty**	Total Gross Weight:	**0,1 kg**
Country	**KAZAKHSTAN**	Volume Weight:	-
Tel/Fax No	**(+7) 727 386 6800**		

Full description of goods	Qty	Unit value	Total value
Two samples of chemicals for analysis;			
Vial 1 : Solid (4 mg)	1	10	10
Vial 2 : In solution (80 mg in 0.28 mL solution)	1	10	10
(see attachment)			
Not IATA-restricted due to Di Minimis Quantities		**Total value for customs**	20
		Currency	NOK

Type of Export Permanent ☒ Repair/Return ☐ Temporary ☐ In Transit ☐

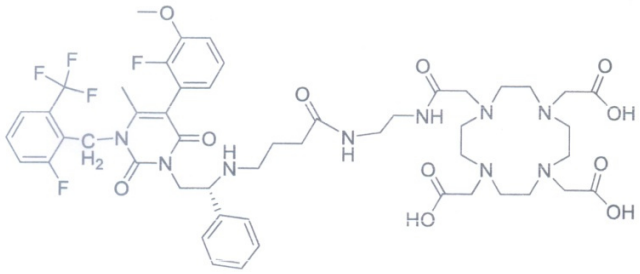
Date June 20th, 2017

Name Runar Staveli

Signature R. Staveli

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF OSLO

RBW025 – Elagolix-DOTA



Chemical Formula: $C_{50}H_{62}F_5N_9O_{11}$
Molecular Weight: 1060,09

Solids

Amount: 4,0 mg
Empty weight flask (with sticker, without cap): 7,8256g

Liquid

About 0,80mg in 0,280 mL solvent (15,5% HCl in H2O/1,4-dioxane; 3:1 (v/v))

If you have any questions, please feel free to contact me.

Good luck!

Best Regards,

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