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SELECTION OF MOBILE PHASE SYSTEMS FOR CHROMATOGRAPHIC RESEARCH OF ^{177}Lu - DOTAELA

Abstract. This paper considered the experimental data on selection of the optimal conditions for separation of free $^{177}Lu^{3+}$cation and labeled ^{177}Lu-DOTAELA complex by paper chromatography in determining the radiochemical purity of the synthesized preparation. The following issues were also reviewed: the effect of structure and the ratio of mobile phase components, and the type of chromatographic paper on separation quality. The most qualitative separation was observed in application of a mobile phase, representing a buffer solution of sodium citrate with pH 5.0 and sodium chloride with the use of the chromatographic paper.

Keywords: paper chromatography, mobile phase, radiopharmaceutical, lutetium-177, DOTAELA.

Introduction. Receptors for triple negative breast cancer express the gonadotropin-releasing hormones (GnRH) in more than 50%cases. Among several analogues (agonists and antagonists) of GnRH that have been studied for treatment of this type of cancer, the non-peptide antagonist elagolix (ELA) is of greatest interest [1].

Selection of ^{177} Lu as a radioactive component of the preparation is determined by optimal depth of penetration in human tissue during radionuclide therapy of small tumors, and also by low radiation load for the healthy organs.

In nuclear medicine, radiopharmaceuticals are used for diagnosis and treatment of oncologic, infectious and other pathologies [2]. Radiopharmaceutical preparations contain the substances labeled with radioactive nuclides. During examination of the particular organ, an appropriate radionuclide labeled carrier substance is used in the body. The resulting complex after administration to the patient accumulates in the particular organ. If the unknown amount of the labeled impurity substance in the radiopharmaceutical finds its way to another organ, the capability of correct evaluation of the patient's examination results can be significantly reduced, especially taking into account a wide range of individual metabolic processes

The decisive factor in obtaining good results is that the radiopharmaceutical has the acceptable radiochemical purity [3]. Determination of radiochemical purity is the only guarantee that a drug, administered to a patient, will provide reliable diagnostics or therapy.

Thin layer chromatography (TLC) and chromatography on paper (CP) are widely recognized as reliable quality control methods in determining of radiochemical purity. These methods enable only one or two of impurity components to be recorded. The quantitative results are very sensitive to the details of testing procedure. TLC and CP methods often require about one hour of analysis time [3]. The United States Pharmacopeia (USP) [4] indicates that minimum 90% of total radioactivity corresponds to radiopharmaceuticals, but the monographs do not indicate the method for radioactivity counting.

The aim of the work is the development of the procedure for determining the radiochemical purity of the synthesized substance of antagonistic action of the triple negative breast cancer DOTA-Elagolix (DOTAELA) labeled with lutetium-177.

The advantages of ^{177}Lu as the therapeutic isotope are determined by its nuclear characteristics: maximum β-energy (maxß = 496 keV, γ = 113 keV (6.4%) and 208 keV (11%)); a half-life of 6.71 days; the optimal depth of penetration into human tissue for radiation therapy of small tumors, a small radiation load on healthy organs [5, 6].

The experiments included the study of the following factors: chromatography paper, mobile phase and its composition.

EXPERIMENT

The chromatographic system was developed for the DOTAELA substance. Radio-labeling was performed with lutetium-177isotope, obtained by irradiating of 200 μg of lutetium chloride, with a thermal neutron flux of 2×10^{14} n/cm$_2$×s for 291 hours. After irradiation, the target was exposed for 6 hours and then transported to the Radiochemical Unit of the Scientific Technical Center of Radiochemistry and Isotopes Production (STC RIP) into the hot chamber for ampoule opening, followed by target dissolution in 2 ml of 0.01 M hydrochloric acid solution.

For DOTAELA radio-labeling, 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 acetate buffer with pH 4.5 was added, then 8 μL of lutetium-177 chloride was added and the volume was adjusted to 2 ml. The final mixture was placed in a glycerin bath at 80-90 °C for 30 minutes. When time was over, the vial was removed and cooled. Then the sample was taken for chromatography.

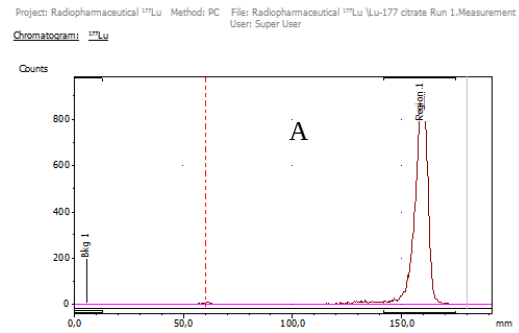
Chromatographic FN1 type paper and Whatman paper No.3 were used as a stationary phase with the total length of 15 cm for the ascending method and 19 cm forthe descending method. Test solution (5 μl)was applied to the start line at 3 cm and 6 cm distance from the start of the chromatographic strip. After application, the spots were dried. The strips were then placed in a sealed chamber, and one end of it was immersed in a solvent [7]. The following was used as mobile phases: 10% solution of ammonium acetate in methanol (30:70 v/v); the solution of sodium chloride 0,9%; 0.1 M sodium citrate buffer solution, pH 5.0.

The analysis was carried out using a radio-chromatogram scanner Veenstra VCS-103 base.

RESULTS AND DISCUSSION

In the course of the chromatograms analysis it was decided to use further the chromatographic paper in the system of solvent descending current due to shorter time of chromatography it provides.

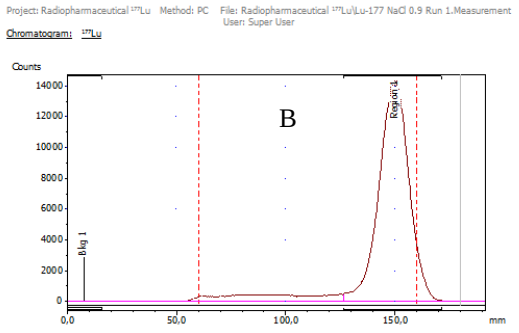
The experimental studies on determination of the optimum conditions for lutetium-177 labeled DOTAELAradiochemical purity showed that this substance is not dissolving in the aqueous solutions. This is confirmed by the chromatograms shown in the figures 1 and 2.



Regions: 177Lu Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Bkg 1	0,0	13,0	-0,453			
Region 1	141,8	174,6	0,823	31148	100,00	96,52
1 Peak				31148	100,00	96,52

Total Area: 32270 Counts
Average Background: 0 Counts

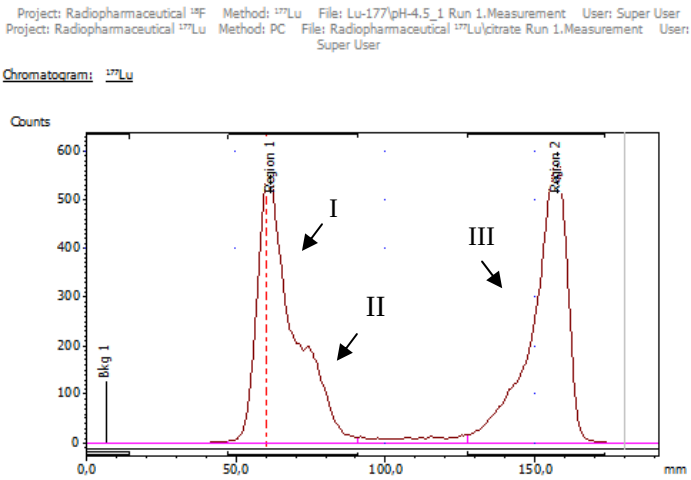


Regions: 177Lu Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Bkg 1	0,0	15,6	-0,520			
Region 1	126,4	171,0	0,902	1141149	100,00	90,33
1 Peak				1141149	100,00	90,33

Total Area: 1263367 Counts
Average Background: 0 Counts

Figure 1 – Chromatograms (A and B) of $^{177}Lu^{3+}$



Regions: ^{177}Lu Detector: PMT

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Bkg 1	0,2	14,6	-0,443			
Region 1	47,4	90,8	0,013	39164	50,29	48,99
Region 2	127,4	173,0	0,813	38705	49,71	48,41
2 Peaks				77868	100,00	97,40

Total Area: 79945 Counts
Average Background: 0 Counts

Figure 2 – Chromatogram of ^{177}Lu-DOTAELA.
Peaks (I): ^{177}Lu-DOTAELA, (II): ^{177}Lu and (III): fragments of ^{177}Lu-DOTAELA radiolysis

In figures 1 and 2, the main ^{177}Lu-DOTAELA peak, detected by the scintillation detector (NaI) after using the aqueous solutions of sodium citrate and sodium chloride as mobile phases, is located on the start line, and the peak, corresponding to the free Lu-177, moves along the chromatogram along with the solvent front. Therefore the important conclusion can be made that ^{177}Lu-DOTAELA does not prevent determination of unreacted Lu-177 impurity in the reaction mixture.

To obtain the chromatograms in figure 3, the chromatographic strip was placed in a system of organic mobile phase of ammonium acetate in methanol. After chromatography in the organic medium, the analysis of the chromatogram in figure 2 showed that the unreacted Lu-177 remains on the start line and is not moving along with the solvent front, while the ^{177}Lu-DOTAELA substance is moving with the solvent front and is not characterized by clear peak character, so this system is not suitable for separation.

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

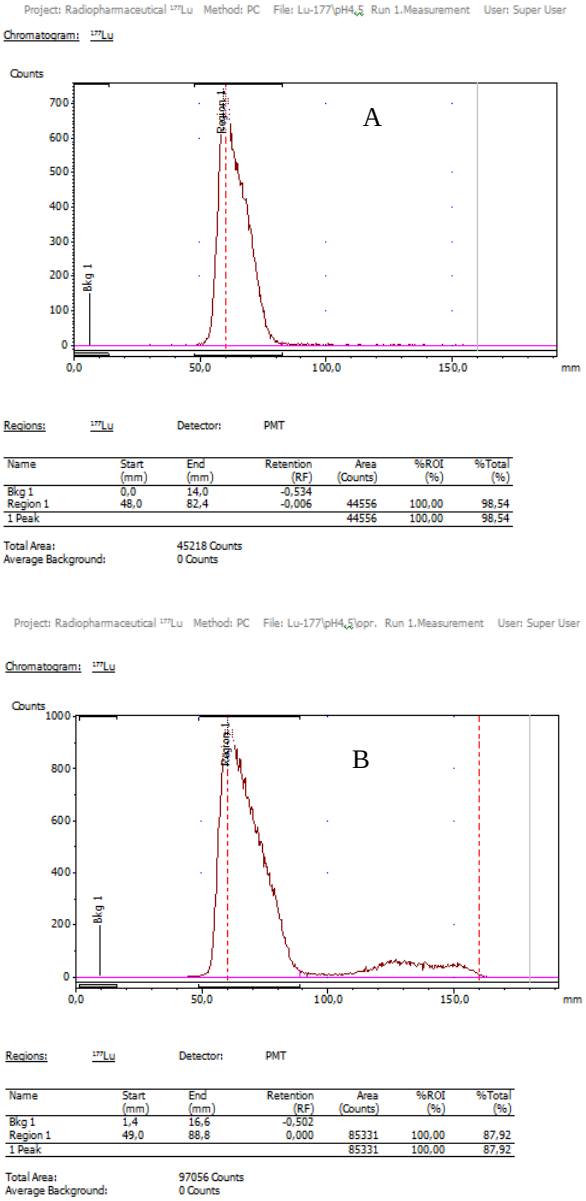


Figure 3 – Chromatograms: A - $^{177}Lu^{3+}$ and B-(I): $^{177}Lu^{3+}$ и (II) ^{177}Lu-DOTAELA

It should be noted that the method of chromatography of synthesis products in two mobile phases of sodium chloride and citrate buffer solution provides an accurate estimate of the content of the radio-labeled target product "^{177}Lu-DOTAELA", as well as the radiochemical impurities of unreacted ^{177}Lu.

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Резюме

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ХРОМАТОГРАФИЯЛЫҚ ЗЕРТТЕУЛЕРГЕ АРНАЛҒАН ҰЯЛЫ ФАЗАЛЫҚ ЖҮЙЕНІ ТАҢДАУ ТАҢБАЛАУ ЛЮТЕЦИЙ-177 ҮШІН DOTAELA

Үш рет теріс сүтбезі қатерлі ісігінің рецепторлары гонадотропинді шығаратын гормондарды (GnRH) 50% істердің саны. Раканың осы түрін емдеуге арналған GnRH бірнеше аналогтары (агонисты және антагонисты) арасында пептидті антагонист элаголикс (ELA) еңқызығу шылыққа ие. Ядролық физика институты Норвегияның Осло университетінің зерттеушілер командасымен жұмыс істейді ^{177}Lu деп белгіленген DOTAELA негізіндегі радиофармацевтика құрылды. Терапиялық изотоп ретінде ^{177}Lu артықшылығы ядролық сипаттамасымен анықталады: кішкентай ісіктердің радиациялық терапиясы үшін адамның ұлпасына енудің оңтайлы тереңдігі, сауоргандарына арналған шағын радиациялық жүктеме. Осы мақалада $^{177}Lu^{3+}$ мен ^{177}Lu-DOTAELA таңбаланған комплексті қағаз хроматографиясымен бөлуге арналған оңтайлы жағдайларды таңдау туралы эксперименталды деректер келтірілген. Сондай-ақ, композицияның әсер етуі және мобильді фазаның құрамдас бөліктерінің бөліну сапасына, хроматографиялық қағаз түріне қатынасы қарастырылды. Натрий цитратының буферлік ерітіндісін ұсынатын мобильді фазаны пайдалану кезінде ең жоғары сапалы бөліну байқалды, pH 5.0 және натрий хлориді.

Түйін сөздер: қағазхроматографиясы, жылжымалы фазасы, радиофармацевтика, лютеций-177, DOTAELA.

Резюме

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ВЫБОР МОБИЛЬНЫХ ФАЗОВЫХ СИСТЕМ
ДЛЯ ХРОМАТОГРАФИЧЕСКИХ ИССЛЕДОВАНИЙ
ДОТАЕЛА ДЛЯ ЛЮТЕЦИА-177

Рецепторы для тройного отрицательного рака молочной железы выражают гонадотропин-высвобождающие гормоны (GnRH) более, чем в 50% случаев. Среди нескольких аналогов (агонистов и антагонистов) GnRH, которые были изучены для лечения этого типа рака, наибольший интерес представляет непептидный антагонист elagolix (ELA). Институт ядерной физики работает с командой исследователей из Университета Осло, Норвегия, в создании радиофармацевтического препарата на основе DOTAELA с маркировкой ^{177}Lu. Преимущества ^{177}Lu в качестве терапевтического изотопа определяются его ядерными характеристиками: максимальной оптимальной глубиной проникновения в ткань человека для лучевой терапии малых опухолей, небольшой радиационной нагрузкой на здоровые органы.

В работе представлены экспериментальные данные по выбору оптимальных условий для разделения свободного катиона $^{177}Lu^{3+}$ и меченого комплекса ^{177}Lu-DOTAELA с помощью бумажной хроматографии. Также были рассмотрены вопросы влияния состава и соотношения компонентов подвижной фазы на качество разделения, тип хроматографической бумаги. Наиболее качественное разделение наблюдалось при использовании подвижной фазы, представляющей буферный раствор цитрата натрия с pH 5,0 и хлоридом натрия.

Ключевые слова: бумажная хроматография, подвижная фаза, радиофармацевтическая, лютеций-177, DOTAELA.