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[1, 3*]A.N. Gurin, [2]Patrick Riss, [1]Ye.T. Chakrova, [1, 3] I.V. Matveyeva

[1]RSE Institute of Nuclear Physics, Ministry of Energy
of the Republic of Kazakhstan, Kazakhstan
[2]University of Oslo, Oslo, Norway
[3]Al- Farabi Kazakh National University, Almaty, Republic of Kazakhstan
[*]e-mail: gurenandrey@mail.ru

Quality control test for ^{177}Lu-DOTAELA

Abstract. This article provides a brief overview of ^{177}Lu-DOTAELA (^{177}Lu -1,4,7,10- tetra-azacyclododecane-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 dioksopirimidin-1-yl]-1-phenylethyl] amino] butanoic acid) quality control based on practical experience. Over the past few years, radionuclide ^{177}Lu has attracted considerable attention and demonstrated great perspectives in the scientific, commercial and clinical communities for application in various therapeutic procedures. Requirements for quality control of radiopharmaceuticals can be found in volume 3 of the State Pharmacopoeia of the Republic of Kazakhstan (SF RK) which is the main regulative document in our country, in the absence of specific monographs and articles in the national Pharmacopoeia it is allowed to use and refer to the Pharmacopoeia of other countries (USA, Japan, UK and Europe). The main requirements for radiopharmaceuticals: pH; radionuclide identification; radiochemical purity; residual solvents; chemical purity; sterility and bacterial endotoxins; checking the integrity of the filter membrane. It should also be noted that there are differences in the quality requirements of some monographs of radiopharmaceuticals in the Pharmacopoeia of different countries. Considering above, during development of the quality control procedure we have to use several regulatory sources. The solution to this problem can be the harmonization of Pharmacopoeia of the Member-States of the Eurasian Economic Union. The process of testing the methodology of quality control is time-consuming and includes many tasks, especially in the area of new radiopharmaceuticals development.

Key words: ^{177}Lu-DOTAELA, radiopharmaceutical, test, quality control (QC), pharmacopoeia, nuclear medicine.

Introduction

The active substance of the radiopharmaceutical being developed for 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 [1]. Elagolix, marketed under the Orilissa brand, which is used to treat pain associated with endometriosis in women. It is also developed for treatment of hysteromyoma and heavy menstrual bleeding in women.

The aim of this work is to highlight the process of developing the quality control procedure, using the example of the developed ^{177}Lu-DOTAELA radiopharmaceutical, which is intended for treatment of triple negative breast cancer.

Materials and metods

Quality control ^{177}Lu-DOTAELA

Quality control of radiopharmaceuticals is one of the most important production stages with the special requirements, established in volume 3 of the State Pharmacopoeia of the Republic of Kazakhstan [2], including in the Pharmacopoeia of the USA [3], great Britain [4] and Europe [5]. The IAEA also published a draft document entitled "Quality Control in the Production of Radiopharmaceuticals" [6]. If we compare the monograph of the same drug in different countries, you can find some differences. The reason for the differences may be the use of different standards. There are tests listed in the BF, but not specified in the GF RK, for example the measurement of radioactivity or USP and BP do not require a test for integrity of the membrane filter.

Table 1 Quality control tests to be performed regularly for the radiopharmaceutical ^{177}Lu-DOTAELA (Fig.1).

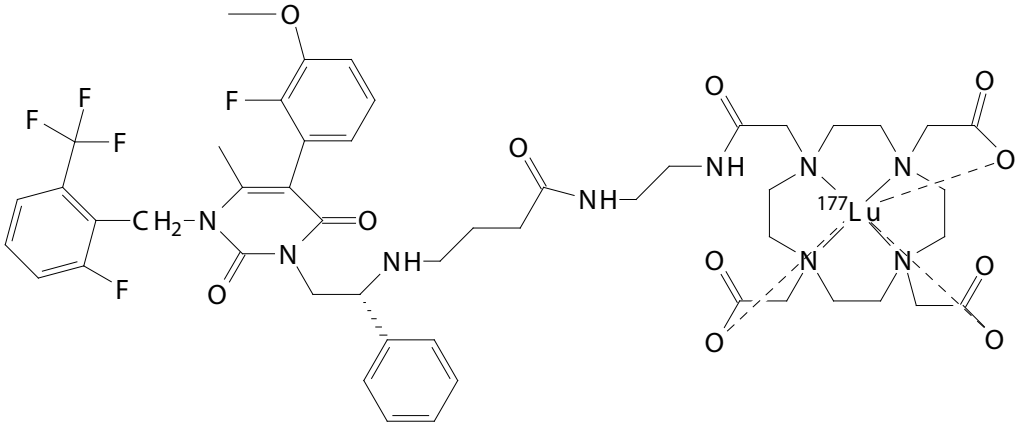

Figure 1 – Structural formula of ^{177}Lu-DOTAELA

Table 1-Quality control analysis ^{177}Lu-DOTAELA

Test	Method	Requirements
Identification ^{177}Lu-DOTAELA	HPLC (SP RK I, vol. 1, 2.2.29)	The relative retention time of 177Lu-DOTAELA should differ by no more than 3 % from the retention time of the non-radioactive Lu-DOTAELA complex.
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, vol. 1, 2.2.3)	from 4.5 to 8.5
Transparency	SP RK I, v. 1, 2.2.1	Transparent compared to *water P*
Color	SP RK I, v. 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	SF RK I, v. 1, *2.9.20*	Mechanical inclusions should be absent.
Sodium chloride	Direct titration	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, vol. 1, *2.9.26)*	Minimum 95%
Residual Solvents	Gas chromatography (SP RK I, vol. 1, 2.2.28)	Ethanol content should be maximum 50 mg/V
Sterility	Direct culture inoculation (SP RK I, v. 2, 2.6.1)	Sterile
Bacterial endotoxins	Turbidimetric kinetic method (SP RK I, v. 1, 2.6.14)	Should not exceed 175/V ME/ml

Results and discussion

Visual analysis methods

If the Pharmacopoeia monographs for radiopharmaceuticals do not specify the test methods, the tests such as transparency, chromaticity and mechanical inclusions are included and mandatory.

In the developed radiopharmaceutical ^{177}Lu-DOTAELA, there should be no mechanical impurities, and it should also be transparent and colorless.

Identity (radionuclidic and radiochemical)

In most cases, the radionuclide and radiochemical identification test is the same test as that for determining the radionuclide and radiochemical purity. In turn, radionuclide identification can be confirmed either by obtaining the gamma spectrum or by measuring the half-life of the product [7].

The half-life measurement can be performed by measuring the same test solution at 2 or more time points. The half-life is calculated using the half-life equation. The SP RK does not specify the time interval between each measurement, but it should be long enough for half-life estimation.

In the SP RK, there are no guidelines for determining radiochemical identification, and according to BP, it can be determined by HPLC or planar chromatography. The planar chromatography method is easy to reproduce, and it is as accurate and reliable as HPLC. However, planar chromatography may require longer time. It should be noted that the results of planar chromatography may vary depending on different grades of plates, chromatographic paper. It is therefore important to use the same grade and freshly prepared mobile phase, if possible. In case when the number of theoretical plates changes with a new batch number (from the same grade), the Rf values shall be confirmed according to the validation process.

Thus, for the tested preparation ^{177}Lu-DOTAELA, the gamma spectrum of ^{177}Lu of the test solution should have a characteristic line with the energy of 0.113 and 0.208 MeV, and the relative retention time of ^{177}Lu-DOTAELA on the chromatogram, obtained by the HPLC method, should differ by maximum 3% from the retention time of the non-radioactive Lu-DOTAELA complex.

pH

The pH of the injection solutions should be as close to the physiological pH as possible. Some laboratories use pH paper to determine the pH of the tested solution, while others use pH meters. It should be noted that the pH indicator paper should be checked using the standard buffer solutions, showing the color change for each pH unit, and the pH value, measured with the pH paper, is approximate.

Residual solvent

Determination of residual solvents is always based on the technological scheme of the drug preparation process. In case of ^{177}Lu-DOTAELA at the purification stage, ethanol is used in the product elution and preconditioning of the Sep-Pak C18 cartridge. Typically, radiopharmaceutical monographs do not specify a method for determining the residual solvents, although the description implies that gas chromatography should be used. The flame-ionization detector is used to determine the residual solvents. Analysis for residual solvents takes only few minutes and 4 minutes for measuring ethanol in ^{177}Lu-DOTAELA. Many laboratories accept ethanol limits of 0.05% or 5 mg/ml, as required by the Pharmacopoeia.

Radionuclidic purity

Radionuclide impurities can form during the production and decay of the radionuclide. Potential radionuclide impurities may be indicated in monographs, and their characteristics are given in General Section 5.7, Table of physical characteristics of radionuclides, mentioned in the European Pharmacopoeia.

In most cases, to establish the radionuclide purity of a radiopharmaceutical, it is necessary to determine the authenticity of each available radionuclide and its radioactivity. Typically, the most common method of evaluation of gamma and X-ray emitters radionuclide purity is gamma-spectrometry. The content of gamma-emitting radionuclide impurities in ^{177}Lu-DOTAELA in total should not exceed 0.1% of total radioactivity. Due to the fact that the build-up of ^{177}Lu is made by "direct" method, which is accompanied by formation of the long-lived isomer ^{177m}Lu($T_{1/2}$= 160 days). In order to reduce the radiation load for the patient and reduce waste activity, the content of ^{177m}Lu in the final product is limited.

Sterility

Sterility should be tested by incubating the tested sample for 14 days at 25 and 37 °C. US FDA recommends the use of a temporary "window" for the analysis of radiopharmaceuticals for sterility, since greater activity in the hours after synthesis can lead to false results. In many cases, the 24-hour window may not be sufficient. Some enterprises send their samples to other microbiological laboratories for sterility testing. The manufacturers shall establish their own protocols in this case.

Bacterial endotoxins

The most common technique for determining the bacterial endotoxins is gel thrombin method, which requires application of amoebocyte lysate (Limulus). When lysate is added to the solution containing bacterial endotoxins, turbidity, precipitation or gelation of the mixture occurs. The analysis usually takes from 25 to 60 minutes.

Spectrophotometric methods are also used to determine the level of bacterial endotoxins. This method is based on changing the color of the substrate due to formation of an enzyme that results from the interaction of endotoxins and lysate. The proenzyme is activated by gram-negative bacterial endotoxins in the lysate, and the concentration of bacterial endotoxins affects the duration of this activation reaction. The cleavage of substrates is activated by a proenzyme, which acts as an enzyme. As a result, there is a change in color of the substrate, which is detected by the spectrophotometric method. The time taken

before the appearance of color change is inversely proportional to the concentration of endotoxin. The endotoxin concentration can be determined by extrapolating the sample reaction time to a standard curve based on the standards containing the known concentrations of endotoxin [8, 9].

The appearance of turbidity by the method of gel-thrombus in the sample can also be the indicator of endotoxins concentration, which is using spectrophotometry. The time of turbidity appearance is inversely proportional to the concentration of endotoxins. The value of endotoxin can be determined by extrapolating the reaction time of the sample to a standard curve constructed using the standards with the known endotoxin concentrations [10]. The appearance of turbidity may be influenced by the presence of polysaccharides such as beta-glucans. Currently, proper methods are being developed to reduce such impacts [11].

Conclusion

Recently, there has been a great interest in development of new radiopharmaceuticals for diagnosis and therapy. Availability of new radiopharmaceuticals and the regulatory framework for application and approval of new medicines creates a new vector for development of nuclear medicine. Synthesis, quality control and production regulation of radiopharmaceuticals such as ^{18}F-FDG, ^{177}Lu-DOTATATE can become the models in development of new radiopharmaceuticals. Volume 3 of the State Pharmacopoeia of Kazakhstan contains some monographs of radiopharmaceuticals, which indicates a positive trend of harmonization with the European Pharmacopoeia. This article provides a brief overview of the quality control of ^{177}Lu-DOTAELA and for those interested in the development of radiopharmaceuticals. However, this article provides only an overview. The interested readers are encouraged to search for more information.

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References

1. Mazen Jamous, Uwe Haberkornand Walter Mier. (2013). Synthesis of Peptide Radiopharmaceuticals for the Therapy and Diagnosis of Tumor Diseases. Molecules, 3379-3409

2. State Pharmacopoeia of the Republic of Kazakhstan, Volume 3, " Zhibek Zholy " Almaty (2014).

3. The United States Pharmacopoeia, 25th ed, and The National Formulary, 20th ed. Rockvile, MD: United States Pharmacopeia Convention Inc, 2002: 752-3.

4. Monographs: Radiopharmaceutical preparation. British Pharmacopoeia 2000. London: British Pharmacopoeia Commission, 2000.

5. European Pharmacopoeia. 4 edition. Strasbourg, France: European Directorate for the Quality of the Medicines, 2002: 1361-8.

6. Quality control in the production of radiopharmaceuticals / International Atomic Energy Agency. Vienna: International Atomic Energy Agency, 2018. | Series: IAEA TECDOC; no. 1856

7. Hung JC. Comparison of various requirements of the quality assurance procedures for (18) F-FDG injection. J Nucl Med 2002; 43 (11): 1495-506.

8. Iwanaga S, Morita T, Harada T, et al. Chromogenic substrates for horseshoe crab clotting enzyme. Its application for the assay of bacterial endotoxins . Haemostasis 1978; 7 (2-3): 183-8.

9. Roth RI, Levin J, Behr S. A modified Limulus amebocyte lysate test with increased sensitivity for detection of bacterial endotoxin . J Lab Clin Med 1989; 114 (3): 306-11.

10. Yokota M, Kambayashi J, Tanaka T, et al. A simple turbidimetric time assay of the endotoxin in plasma. J Biochem Biophys Methods 1989; 18 (2): 97-104.

11. Kambayashi J, Yokota M, Sakon M, et al. A novel endotoxin specific assay by turbidimetry with Limulus amoebocyte lysate containing beta-glucan. J Biochem Biophys Methods 1991; 22 (2): 93-100