CHARLES UNIVERSITY IN PRAGUE, FACULTY OF PHARMACY IN HRADEC KRALOVE,
Department of Biophysics and Physical Chemistry

STUDY OF RADIOLABELING

OF CHEMICALLY MODIFIED BOMBESINES

Master Thesis

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I declare that this thesis is my original authorial work, which I developed independently. All literature and other sources from which I drew during processing are listed in the bibliography and are properly cited. The work was not used to obtain the same or a different degree.

In Patras on 10th May 2013 .....

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1. Introduction and the aim of the study

One of the most important part in pharmacy is radiopharmacy, a sector which is dealing with the development and production of radioactive drugs. Nuclear medicine in cooperation with radiopharmacy has developed a very promising approach in diagnosis and therapy as far as is concerned oncology, the so called “molecular targeting”.

This idea lies in the development of drugs for cancer which can effectively help in treatment of the diseased tumorous tissues and protect at the same time side effects of drugs on the healthy ones. Radiolabeled molecules possess a great potential as tracers for metabolic pathways and organ function *in vivo.*

Surgery, chemotherapy and external beam radiation were the main therapeutic methods in the treatment of human cancers in the last 50 years. Because of very low tumour selectivity of chemotherapeutics, systemic chemotherapy is often limited by serious side effects to normal tissues. A consequence of these side effects is the use of suboptimal doses which may results in therapeutic failure and also the development of drug resistance.

For this reason, the development of ligands for targeted radiotherapy of cancers, including drug-ligand conjugates has become a major goal in recent years. The strategy to improve therapeutic selectivity is to couple therapeutic agents to vectors such as monoclonal antibodies, their fragments, or smaller molecules such as amino acids or peptides. In targeted radionuclide therapy, the cytotoxic part of the conjugates contains mostly therapeutic radiometal bound to a bifunctional chelator.

When choosing an appropriate vector that direct the radiotherapeutical to the target the knowledge of both its pharmacokinetic behavior, as well as the availability is important. Peptides against antibodies have an advantage of faster pharmacokinetics and significantly lower production costs.

The aim of the study

- ***a)*** To study the literature that relates to radiolabeled receptor-specific peptides from a group of bombesin derivatives and mechanism of action on which the diagnostic and therapeutic use of labeled derivatives is based.
- ***b)*** Focusing on bombesin derivative DOTA-Lys3-bombesin, which was synthesized in the Nuclear Physics Institute in Rez to find the optimum conditions for radioactive labeling of the peptide with therapeutic radionuclide lutetium-177 in the required purity for biodistribution studies at least 97%.
- ***c)*** Using standard methods of analysis of radiolabeled peptides to determine the stability of the labeled product.

2. Theoretical part

2.1 Cancer

Cancer is one of the most serious health problems seen today in developed countries. Statistics show that it is the second most common cause of death after heart diseases. Usually it affects older people, but there are cancers that occur in younger people as well, even children.

The term "cancer" is not attributable to a single disease but a group of diseases characterized by uncontrolled cell proliferation. Unlike normal cells in our body, which grow, divide, and die in a strictly controlled manner, tumor cells differ because they continue to divide uncontrollably. This has resulted in the development of a cell mass, called a tumor. Tumors can be benign or malignant.

Prostate cancer is a type of cancer that develops in the prostate gland of the male reproductive system. Prostate cancer seems to appear in men over the age of 50 years old. It appears to be the sixth (related with cancer) cause of death worldwide, in men. For this reason many researches have began in order to eliminate this situation.

Number of common human tumors, including those of prostate, breast and lung cancer, express increased levels of the gastrin-releasing peptide receptor (GRP-R), which means that this receptor is a potential target for peptide receptor and targeted radionuclide therapy.[1-13]

2.2 Bombesin origin

Bombesin (BB) is a linear tetracedapeptide with sequence Glu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH2, first isolated from the skin of oriental fire-bellied toad (*Bombina orientalis*) and exhibit high affinity in binding and speciality release with gastrin receptors (GRP-RS).

It has two major analogs known as neuromedin B (NMB) and gastrin- releasing peptide (GRP), which handle a variety of physiological actions in nervous system and gut, such as secretions of adrenal, pituitary and gastrointestinal hormones. BBN-like peptides induce their effects after binding to members of G-protein-coupled receptors. There are 4 kinds of these receptors which are located at the outer membrane of target cells:

a) GRP-R [gastrin-releasing peptide receptor]

b) NMB-R [neuromedin B receptor]

c) The orphan receptor bb3-R and

d) The amphibian receptor bb4-R [which occurs a high affinity for all the other receptor subtypes].

The main role of these bombesin-like peptides has been described in numerous studies because of their significance.[2, 4-6, 13] Especially tumor gastrin-releasing peptide (GRP) receptors are potential targets for therapy and diagnostic purposes due to their efficacy as molecular carriers for cytotoxic drugs or radionuclides and high overexpression of corresponding receptors which have been detected usually in edrocrine- related cancer cells and in vascular bed of some tumors.

2.3 Modification of peptides for labeling with metallic radionuclides

The development in anticancer therapy as far as is concerned the drug-ligand conjugates has been evaluated the last few years, due to the lack of selectivity in systemic cytotoxic chemotherapy and the serious side effects that occur during the therapy.

One new approach to improve this kind of problem is to couple therapeutics to vectors, like monoclonal antibodies, or smaller molecules (like regulatory peptides).

Bombesin receptors (gastrin-releasing peptides) are very important because they are overexpressed in various and serious types of cancer such as prostate, breast and even lung cancers. These gastrin- releasing peptides could be used as carriers for delivering cytotoxic drugs or therapeutical radionuclides into tumor cells or diagnostic radionuclide to visualize the region of interest.

Furthermore to select a proper receptor binding sequence and the suitable radionuclide, a certain synthetic and labeling strategy must be followed in order to prepare hydrophilic conjugate, eliminated through the renal system and not by the hepatobiliary or gastrointestinal tract, so that we'll have the less uptake in non-target tissues.

In targeted radionuclide therapy peptides are radiolabeled with hard Lewis acid bi- and trivalent lanthanides and similar radiometals (^{90}Y, ^{177}Lu, ^{111}In,..). For this reason bombesin derivatives afford the binding of very hydrophilic radiotracers. Bifunctional chelators such as DTPA (=diethylenetriaminepenteacetic acid) or DOTA (=1,4,7,10-tetraazacyclododeca-ne-N,N',N'',N'''-tetraacetic acid) are coupled at the N-terminus of the peptides.[14]

The radiotherapy of neuroendocrine tumors, mainly gastrointestinal cancers through sst (Somatostatin) receptors may be achieved by labeling of DTPA-octreotide (DTPA-OC) with Y-90, a therapeutic radionuclide that emits beta radiation of long range. As it is known, the complex

of yttrium with DTPA is not particularly stable *in-vivo*, thus conjugated octreotide analogs with the chelator DOTA were developed forming very stable complexes with trivalent metals. When coupling one amidated carboxylic acid of DOTA to the N-terminus of the D-Phe1 peptide. There is also tyrosine instead phenylalanine at position 3 of the peptide, DOTA-Tyr3-octreotide (DOTA-TOC). This substitution increases the hydrophilicity of radiopeptides and surprisingly also affinity to somatostatin receptors.

Another modification can be done by replacing threonine-ol at position 8 in carboxylic terminus with threonine and resulting DOTA-Tyr3-octreotate (DOTA-TATE) labeled with therapeutic radionuclides exhibited further higher proportion of internalization in tumor cells.

Both of modified peptides, DOTA-TOC and DOTA-TATE, have been labeled with Y-90 and Lu-177, with incubation at pH 5.5, at 90° C, for 20 min. Y-90 is widely used in nuclear medicine to treat tumors of larger volumes due to its greater energy of beta emission (2.28 MeV) and thus a greater range in tissue, while the Lu-177 of minor beta energy (490 keV) is effective in smaller tumors. [15,16]

2.4 Bifunctional chelators -BFC

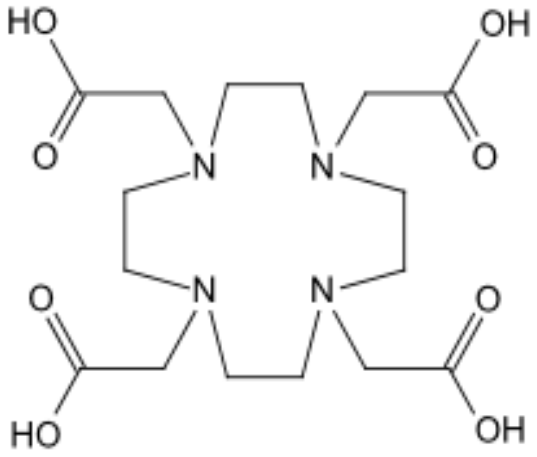
The metal complexes with polydentate ligands are typically called helates (chelates) from the Greek word "χηλή" (caliper).

The choice of chelating system for biomedical imaging and therapeutic applications is largely determined by the nature of the metal ion required for the given application. Several rare earth metal ions (lanthanides and yttrium) and main group III elements are frequently selected for diagnostic (^{111}In, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{177}Lu) and/or therapeutic (^{90}Y, ^{177}Lu, ^{166}Ho, ^{149}Pm, ^{153}Sm) nuclear medicine applications.

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)

It is an organic compound which is used as a complexing agent especially for lanthanide ions.

DOTA is derived from the macrocycle **cyclen** (1,4,7,10-tatraazacyclododecane) and has high affinity in binding with bi- and trivalent cations. As a polydentate ligand the bonds formed with the cations depend on the geometric tendencies of the metal cation.



Ilustration 1: DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)

DOTA conjugated by one of the four carboxyl groups as an amide to terminal amino-group of peptide structure is used as a chelating agent to bind diagnostic or therapeutic radionuclides. For that reason is considered to be the most widely used chelator for radiolabeling in radiopharmacy.

Main advantage of this chelator is high thermodynamic stability of its complexes, but the drawback is slow kinetics of the chelate formation which requires high reaction temperature up to 100°C, which is unsuitable when thermally sensitive biological structures (antibodies or unstable peptides) are radiolabeled.

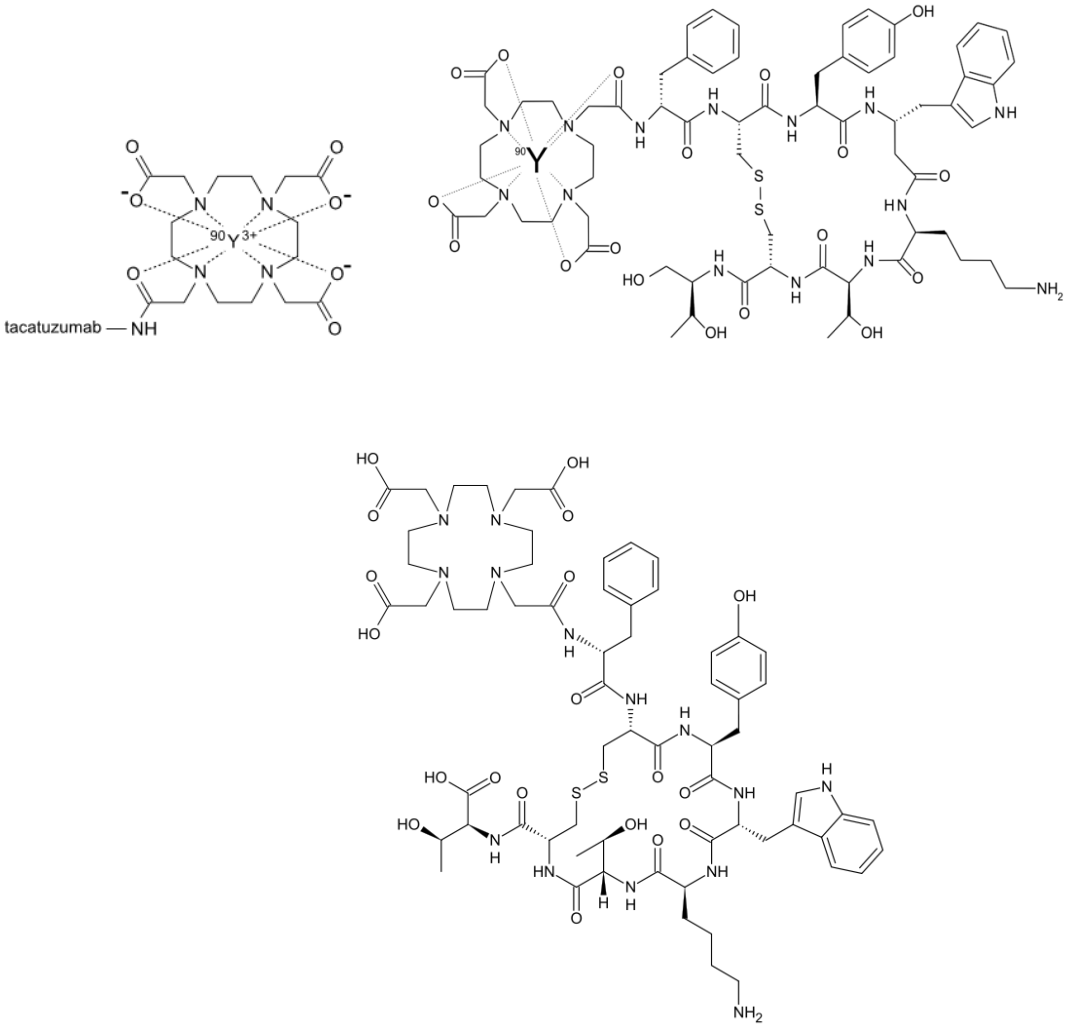


Illustration 2: Structures of Y-DOTA linked to the monoclonal antibody tacatuzumab, Y-DOTA-TOC and cold DOTA-TATE [17]

DTPA (diethylene triamine pentaacetic acid) "pentetic acid"

It is a polyamino-carboxylic acid consisting of a diethylenetriamine backbone with five carboxymethyl groups. It's similar to EDTA molecule. The chelator has also high affinity in binding with metal cations. As an octadentate ligand it has the formation constant for its complexes about 100-times greater than those for EDTA.

As a chelating agent, DTPA wraps around a metal ion by forming up to eight bonds. Transition metals, however, usually form less than eight coordination bounds. So, after making the complex, DOTA still has the ability of binding with other reagents using three amine centers and four of five carboxylic groups for complexation. [18]

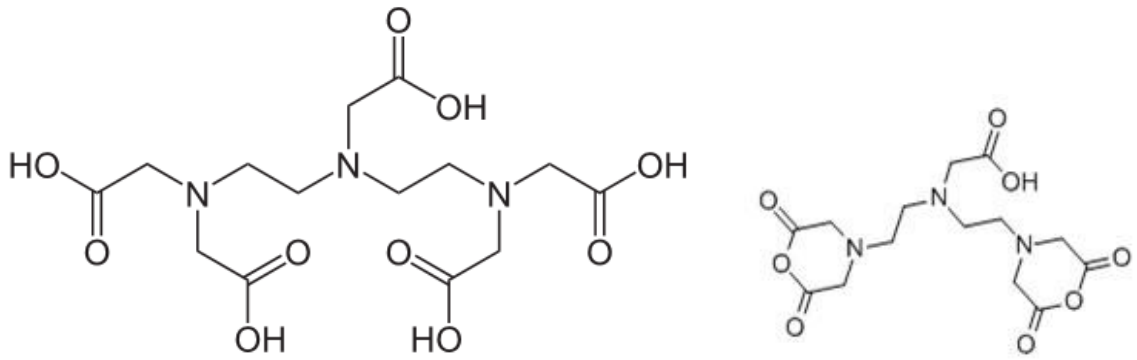


Illustration 3: Diethylene triamine pentaacetic acid (DTPA) with it's anhydride

NOTA (1,4,7-triazacyclononane-N,N',N''-triacetic acid)

NOTA has a smaller ring structure in comparison to DOTA and only has 3 chelating carboxylate groups and 3 nitrogens, it poses 6 coordination sites with metal ions.

Contemporary it is studied as potential chelator for Cu-radiometals. [19]

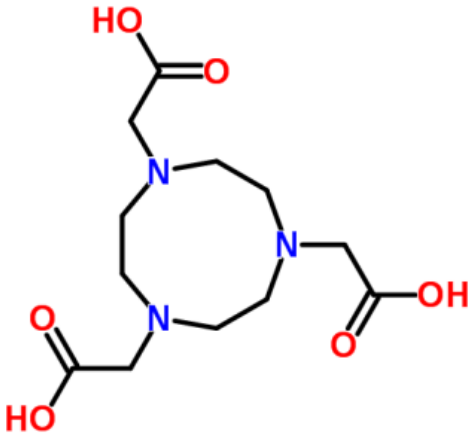


Illustration 4: 1,4,7-triazacyclononane-N,N',N''-triacetic acid

TETA (1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid)

TETA has 4 chelating carboxylate groups and 4 nitrogens. C- and N-functionalized derivatives can be prepared with TETA. A p-bromoacetamidobenzyle-TETA derivative could be used to modify antibodies through sulfhydryl groups. The chelator was used for modification of somatostatin derovative octreotide and its labeling with ^{64}Cu. [20]

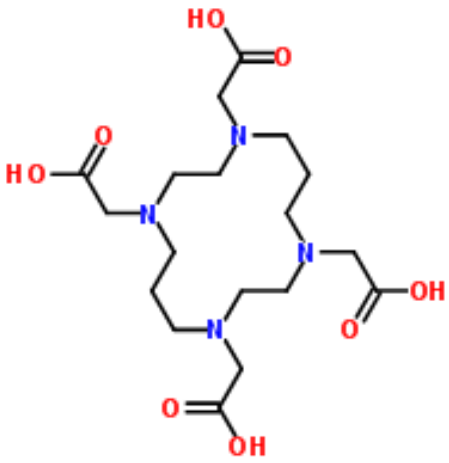


Illustration 5: 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid.[21]

HYNIC (Hydrazino nicotinic acid)

The complex is preferentially used for labeling proteins with radionuclides of Tc or Re

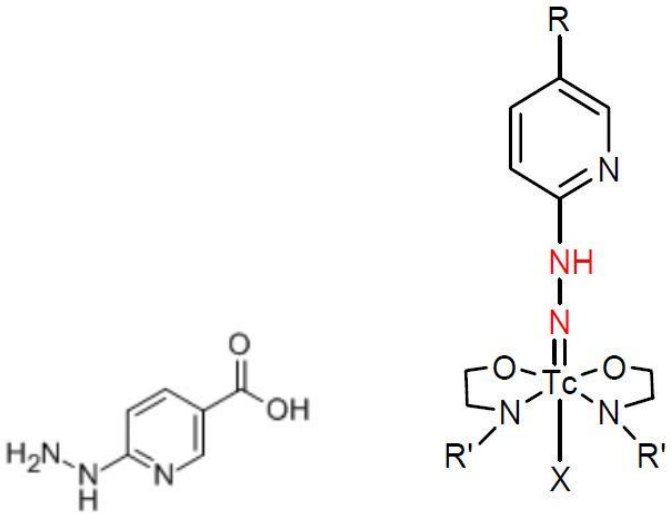


Illustration 6: HYNIC and its complex with ^{99m}Tc.[22]

2.5 Radionuclides

Radionuclides are atoms of which their nucleus is possible to transform spontaneously into nucleus of other nuclides, stable or radioactive. This transformation can include the release of electric charged particles (α and β^+ or β^-), the regional electron capture with simultaneous x-ray emission from the peripherals of the individual layers of the atom or the emission of gamma rays. Radionuclides can occur naturally, or can be produced artificially.

A radionuclide for successful therapy must have specific physical properties, such as half-life, particle emissions, availability etc., also it requires the necessary ligand framework to stabilize the metallic radioisotope *in vivo,* and the relative ease in which the radio-conjugate is prepared.[14,23]. The most proper elements for radiotherapy are the radiolanthanides and lanthanide-like ones, and this is because of their natural properties of forming identical structures in aqueous solutions.

Technetium-99m, (^{99}Tc)

Tc-99m is a widely used diagnostic radioactive tracer in nuclear Medicine, with excellent physical parameters (Eγ-emission 140 keV) for contemporary scintigraphic techniques.

The short half-life, both biological and physical, leading to a very fast elimination from the body after imaging process allows us to collect data rapidly without exposure the patient to a high radiation levels. These reasons make Technetium-99m suitable for diagnostic purposes.[24]

Yttrium-90, (^{90}Y)

It is a medical isotope of yttrium, also widely used in medicine for the treatment of several types of cancer. ^{90}Y with half-life 2,67 day and maximum beta emission $E_{\beta max}$ = 2.27 MeV seems to be one of the major isotopes for targeting radiotherapy. However some disadvantages keep it away from the title of the “ideal radionuclide”, its particle range of ~12mm limits its ability especially for larger tumors volumes.[25]

In addition the high energy β^- of ^{90}Y could be also a cause for renal toxicity in patients treated with new ^{90}Y-DOTATOC radiopharmaceuticals.[25, 26]

Indium-111, (^{111}In)

Indium-111 decays by electron capture to stable cadmium-111, emitting a 0.1713 and 0.2454 MeV gamma rays with half-live 2.8047 days. In medicine it is used mostly for diagnosis but high doses of radionuclide were also tested for therapy due to accompany by low energy Auger electrons in decay of radionuclide.[27-29]

The Auger effect is a physical phenomenon in which the filling of an inner-shell vacancy of an atom is accompanied by the emission of an electron from the same atom. When a core electron is removed (by electron capture), leaving a vacancy, an electron from a higher energy level may fall into the vacancy, resulting in a release of energy. Although sometimes this energy is released in the form of an emitted photon, the energy can also be transferred to another electron, which is ejected from the atom. This second ejected electron is called an Auger electron**,** after one of its discoverers, Pierre Victor Auger. [30]

Lutetium-177, (^{177}Lu)

It's the medical radioisotope of the last element in the Lanthanide series, Lutetium. Radionuclide ^{177}Lu decays with half-life 6.71 days with emission β^- particle of energy maximum $E_{\beta max}$= 0.497 MeV.[31]

Due to these characteristics it seems to be one of the most favorable radionuclide used in radiotherapy. Furthermore its particle range is ~ 2mm which makes it suitable for therapy of smaller tumors. Also the presence of a low-abundance 208 keV gamma photon allows for *ex vivo* development of the *in vivo* targeting availability of the administered ^{177}Lu biomolecular targeting agent.[32]

Peptides which are labeled with this radionuclide have been shown less damaging in healthy tissues because of the short β-particle range of ^{177}Lu and the possibility that offers us to use one radiolabeled agent for both diagnosis and therapy.[6,25,26]

Galium-67, (^{67}Ga)

Gallium-67 citrate is produced by a cyclotron. Charged particle bombardment of enriched Zn-68 is used to produce gallium-67. The gallium-67 is then complexed with citric acid to form gallium citrate. The half-life of gallium-67 is 78 hours. It decays by electron capture, than emits de-excitation gamma rays that are detected by a gamma camera. Photopeaks: Energy Abundance: 93 keV 40%, 184 keV 20%, 300 keV 17% and 393 keV 5%.
Mechanism of distribution: The body generally handles Ga^{3+} as though it were ferric iron (Fe-III), and thus the free isotope ion is bound (and concentrates) in areas of inflammation, such as an infection site, and also areas of rapid cell division. Gallium (III) (Ga^{+3}) binds to transferrin, leukocyte lactoferrin, bacterial siderophores, inflammatory proteins, and cell-membranes in neutrophils, both living and dead. This relatively nonspecific gallium binding allows sites with tumor, inflammation, and both acute and chronic infection to be imaged by nuclear scan techniques.[33]

Galium-68, (^{68}Ga)

The positron-emitting isotope Gallium-68 is a generator radionuclide. A gallium-68 generator is a device used to extract ^{68}Ga gallium from a source of decaying germanium-68. The parent isotope ^{68}Ge has a half-life of 271 days and can be easily sent to hospitals within the generator, where it is storable for almost a year. Its decay product gallium-68 (with a half-life of only 68 minutes, inconvenient for transport) is extracted and used for certain positron emission tomography nuclear medicine diagnostic procedures, where the radioisotope's relatively short half-life and emission of positrons for creation of 3-dimensional PET scans, are useful.[34]

2.6 Diagnosis and therapy

Radiation therapy works by damaging the DNA of the tumor cells. This damage in the DNA may be caused by two types of radiation, photon or a charged particle. It can be done by direct or indirect ionization of the particles which are parts in the DNA's chain. Indirect ionization happens as a result of the ionization of water forming free radicals and especially hydroxyl radicals, which are going to damage the DNA.[35]

Beta decay

Beta decay (β decay) is a type of radioactive decay in which a beta particle (an electron or a positron) is emitted from an atomic nucleus.

There are three types of beta decay, a *beta minus*, *beta plus* and *electron capture*. In the case of beta decay that produces an electron emission, it is referred to as beta minus (β^-), while in the case of an emission of positively charged electron as beta plus (β^+).

Nuclei which decay by positron emission may also decay by an electron capture. For low-energy decays, electron capture is energetically favored. In this type of decomposition positron is trapped in the surrounding material and by its interaction with negatron annihilation of matter occur with two gamma quanta irradiated.[36,37]

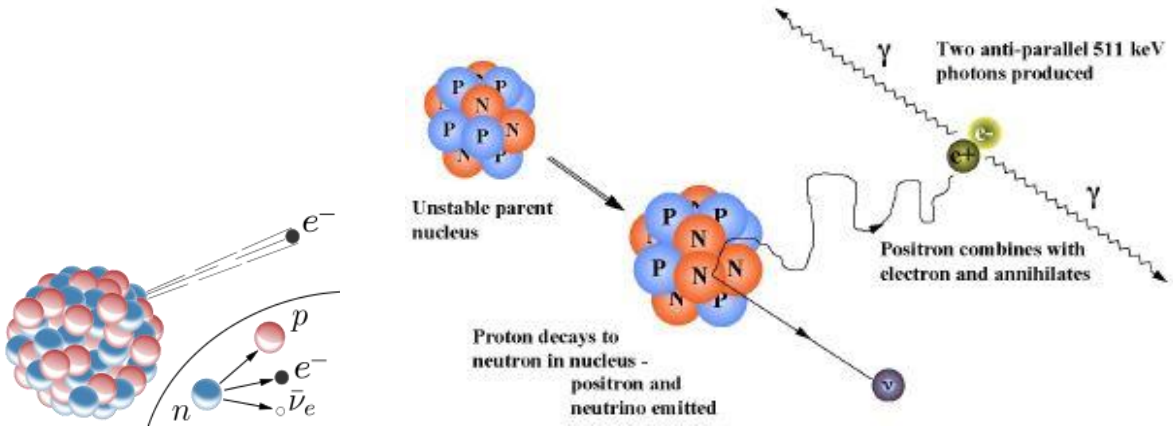


Illustration 7: Scheme of beta- and beta+ decays.[36,38]

What we call display?

Display call taking pictures and evaluation of internal and external human organs obtained by special machines that use different forms of energy (X-rays, electromagnetic radiation, gamma rays). The display is not a cure but helps the doctor to take the necessary decisions.

PET- Positron Emission Tomography

It is a diagnostic test based on the detection of radiation from the emission tomography. *Positrons* are tiny particles emitted from a radioactive substance administered to the patient. Commonly used to detect tumors and to determine their extent and for monitoring the effectiveness of antineoplastic therapy through the characterization of biochemical changes (especially glucose metabolism) of the tumor. Detects cancer, controls blood flow and sees how work the organs. By combining CT and PET (PET/CT) one can distinguish normal from abnormal tissues. The technique is more accurate in detecting larger than 2 cm and for more aggressive tumors,

To PET/CT more accurate than PET in the staging of tumors and accurately detect. It can also be used to assess the effectiveness of a therapy - detecting tumor cells die and thus use less glucose. The radiation exposure is small and does not affect the normal functions of the body. Pregnant patients should inform their doctor before they are submitted to an examination to assess risks compared with the benefits.[39]

SPECT - Single photon emission computer tomography

In this type of imaging radioactive probes were used and a scanning system data transferred to the computer to form two and three dimensional images. It's using gamma rays and provides us a true 3D information.

The basic technique includes the delivery of a radionuclide which is gamma-emitting radioisotope. This procedure undergoes usually via injection through the bloodstream of the patient and the radionuclide is made to be attached only to a characteristic ligand in certain types of tissues. After that this complex transferred and bound to a site of interest in the tissue and due to the gamma-rays properties that the isotope has allow us to visualize the radioligand concentration with use of gamma cameras. [40]

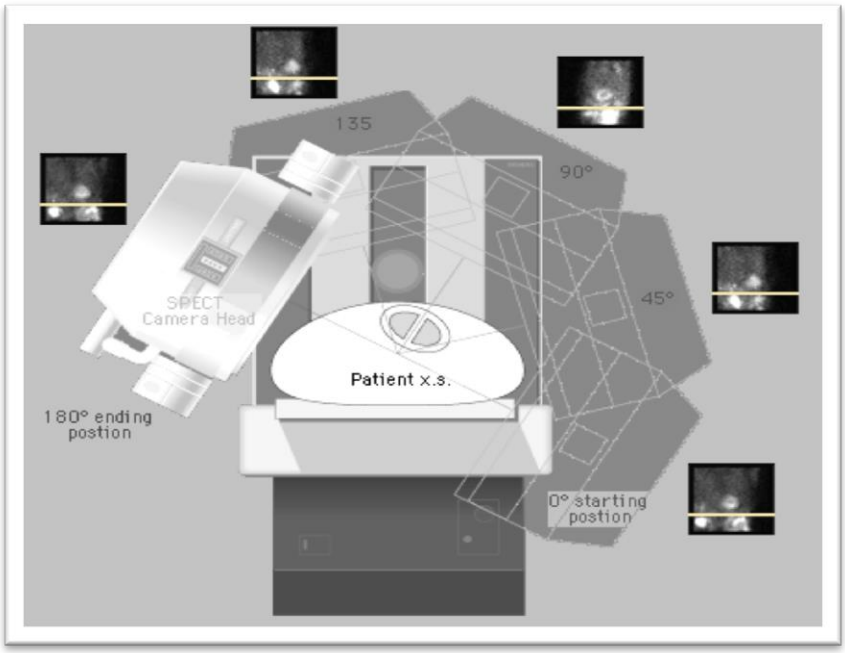


Illustration 8: SPECT machine performing a total body bone scan. The patient lies on a table that slides through the machine, while a pair of gamma cameras rotate around her.[41]

2.7 Analytical Methods

HPLC – High Performance Liquid Chromatography

High performance liquid chromatography, also known as High Pressure Liquid Chromatography and commonly abbreviated as HPLC, is a form of chromatography systems used frequently in biochemistry and analytical chemistry.

The HPLC technique can be used for separations based on adsorption, distribution, ion exchange and molecular exclusion, while for each application are available from various manufacturers appropriate columns.

With the development of HPLC technics separation times decreased significantly. In this technique, the filling material of the column is of fine partitioning (particles of very small diameter) and has a high spherical regularity so as to achieve a high degree of uniformity and packing density. The high packing density with these very small particles reduces the flow rate of mobile phase through the column (large transfer resistance) and to achieve a reasonable flow rate required application of high pressure in the mobile phase. The basic parts of an HPLC apparatus are presented on Illustration 9: 1) a system for mobile phase 2) sample introduction system, 3) column, 4) detector and 5) recorder.

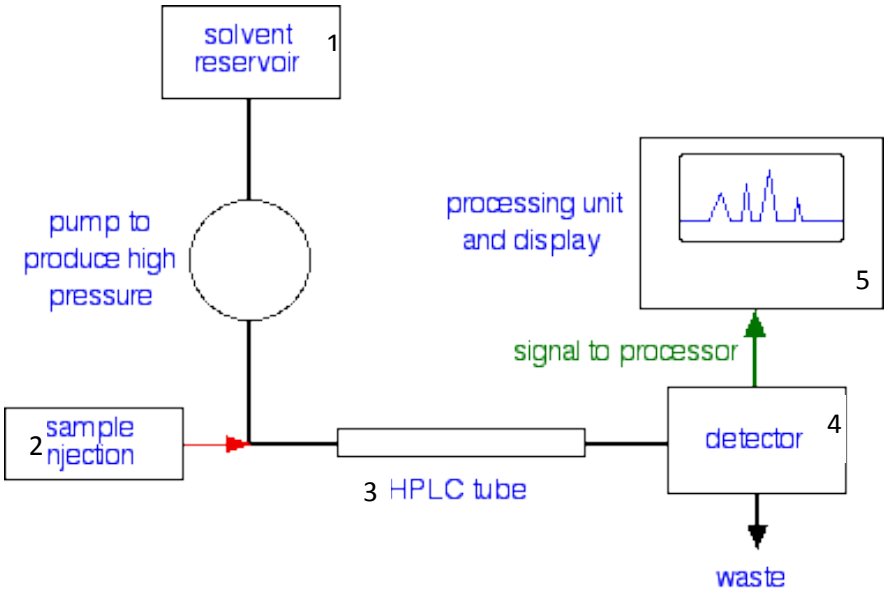


Illustration 9: A flow scheme for HPLC [42]

TLC – Thin Layer Chromatography

In this chromatographic technique glass or aluminum plates coated with a thin layer of static phase is used. In stationary phase is usually silica gel or aluminum oxide less often, cellulose etc.

The solution of the test sample is placed under the form of a spot in the top of the plate at a distance of about 2 cm. Then the plate is placed upright in an airtight chamber in which has been introduced a suitable solvent system at a height lower than that of the spot. The solvents must be placed within the chamber at least 10 min before the placement of the plate is saturated to the headspace of the solvent vapors. The solvent was then allowed to rise with the aid of capillary (about 10-20 min, depending on the height of the plate) until the solvent front reaches a few centimeters before the end of the plate. Then, the plate was removed and dried with a stream of air.

The various substances in the test sample on the plate moving at different speeds depending on their polarity and appear as discrete spots.

The observation of spots made by examination under UV light (254 or 356 nm) or by spraying with specific reagents. From the color of the spots in the visible, the ultraviolet absorption, depending on the reagent used can draw conclusions about the class of substances that we observe (flavonoids, alkaloids, sugar etc). For the detection of radioactive substances a variety of radiation detectors are usually used depending on the nature of the radionuclide.

3. Experimental part

3.1. Materials

Chemicals:

Radionuclide used: Lu-177: 10 mCi in the form of $LuCl_3$ solution in 0.05M HCl, volume 8µl, specific activity 5 Ci/mg at expiry, producer PerkinElmer Life and Analytical Sciences, Boston, MA, USA.

Peptide tested: Lys3-bombesin modified with chelator DOTA (Illustration 10) was prepared at Institute of Nuclear Physics of Czech Academy of Sciences at Řež near Prague by Ing. Miloš Beran, CSc. within collaboration in project EUREKA Grant No.E08018 of the Czech Ministry of Education. Lyophilized peptide was delivered in an amount 200 µg in the penicillin vials closed by a rubber stopper without closing by metal lid and stored at -20°C.

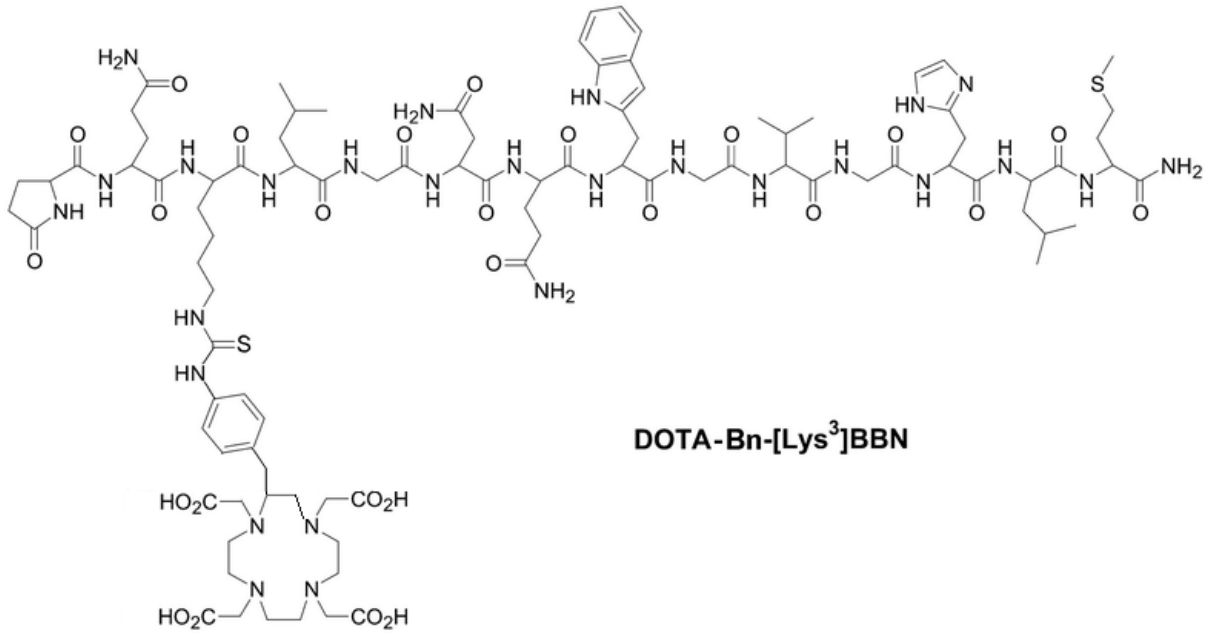


Illustration 10: Structure of the peptide studied

Usual Chemicals:

CH_3CN for HPLC. (Sigma)

CF_3COOH p.a. (Sigma)

CH_3COONa, super pure (Merck)

CH_3COOH, p.a. (Merck)

Sodium chloride, 0.9% solution (Braun)

EDTA (Ethylenediaminetetraacetic acid) (Sigma)

DTPA (Diethylenetriaminepentaacetic acid) (Sigma)

Water was purified by reverse osmosis MilliQ-quality (system Millipore)

0.4 M acetate buffer with 0.24 M gentisic acid pH = 4.52

Thin Layer - ITLC-SG (instant glass fibers filled with silica gel) 5x17 cm, producer Pall or Gelman Sciences, USA

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For the purpose of rapid analysis (lasting a few minutes) in the radiopharmacy are usually used rapidly rising layers - ITLC-SG (Instant Thin Layer Chromatography Medium (ITLC) which is a binderless, glass microfiber chromatography layer impregnated with a silica gel (SG). It is a porous, slightly acidic layer that provides excellent resolution)

3.2. Instrumentation

- Gama-automat Wizard, Finland

- TLC Radio chromatograph, Raytes with ionization chamber and evaluation programs RITA STAR and GINA STAR

- HPLC Agilent System 1100 Series with UV and radio-detection, with scintillation probe and Radiometer fy Polon, Type URL 2 and registration with chromatographic station Data Apex, column: Lichrocart Lichnosphere 100 RP-18e 3x150 mm, grain 5µm

- Heating plate for tubes Eppendorf

3.3. Methods

3.3.1. Determination of the purity of the cold peptide studied

Purity of the peptide studied was determined by HPLC analysis on the Agilent System 1100 Series with UV detection at a wavelength of 240 and 280 nm and with gradient elution. Conditions: Mobile phase A: 0.1% TFA in water, mobile phase B: 0.1% TFA in acetonitrile, flow rate 1 ml / min, t = 25 ° C with gradient presented on Table 1:

Table 1: Gradient of the mobile phase in HPLC analysis of unlabeled peptide (ABX, Radeberg Ge)

Time	Composition of the mobile phase
0 - 35 min	0 – 100% B

3.3.2. Radiolabeling of the peptide

First, it was necessary to find out the reaction conditions for the radiolabeling of structures near to bombesins: pH, temperature and the heating time. For this purpose, we came out from the method of von Guggenberg with coworkers who studied radiolabeling of different minigastrines modified with the same chelator - DOTA.[43]

Radiolabeling of the peptide was carried out in polypropylene 1.9 ml Eppendorf tube:

To 100 µl 0.4M acetate buffer pH 4.54 5 µl of the peptide solution was added (corresponding to 5 µg of the peptide) and after that 0.6 µl of ^{177}Lu solution corresponding to activity of the radionuclide 0.75 mCi. The reaction mixture was heated first for 20 minutes at 80° C (reaction conditions found for radiolabeling of sensitive minigastrins). After 15 min standing, the analysis of radiochemical purity of the labeled product was carried out by HPLC analysis and/or by thin-layer chromatography on ITLC-SG described by Sosabowski and Mather who studied a modification of different peptides with chelator DOTA and examined reaction conditions of their radiolabeling with trivalent radiometals. [44]

3.3.3. Thin layer chromatography

For the analysis rapidly evolving ITLC-SG strips (glass fibers with silica gel) were utilized with dimensions of 0.5 x 8cm (Gelman Sciences Company or PAL) in several mobile phases:

a) 0.1 M ammonium acetate water solution with 10 mM EDTA pH 5.5: In this system only free form of radiometal moves with the front of the mobile phase, other forms remain at the origin. [44]

b) 10% (w/v) ammonium hydroxide water solution with methanol 1:1 (v:v): In this system the labeled peptide or low molecular weight complex move with the front of the mobile phase and free form or hydrolyzed radiometal remain on the origin. [44]

A small volume (0.5 - 1 µl) of the sample was applied to the strip, after the development (which lasted for about several minutes) and drying up chromatogram was analyzed on radio-chromatograph Rita Star.

3.3.4. HPLC analysis

Radiochemical purity of ^{177}Lu-DOTA-Lys^3-bombesin was determined by HPLC analysis on the Agilent System 1100 Series with radiodetection and with gradient elution. Conditions: Mobile phase A: 0.1% TFA in water, mobile phase B: 0.1% TFA in acetonitrile, flow rate 1 ml / min, injection volume 10 µl, $t_{at\ analysis}$ = 25 ° C with gradient:

Table 2: Gradient of the mobile phase in HPLC analysis of radiolabeled peptide

Time of elution	složení mobilní fáze
0 – 6 min	0%B
6 - 15 min	0-100% B
15 - 20 min	100%B
20 - 25 min	100-0%B

Sample preparation for an HPLC analysis: to 10 µl of mobile phase A 2 µl of the sample together with 3 µl of 10^{-3} M DTPA solution (pH 5) was added. DTPA is weaker chelator than DOTA, so there is no trans-chelation of radionuclide from labeled peptide, but it protects column against contamination with free radiometal that may be present in the sample. The free radiometal appears at the beginning of the record as a DTPA-complex at time around the 4th minutes.

Tab. 3: Study of the labeling conditions on quality of ^{177}Lu-DOTA-Lys^3-bombesin: dependence on pH, time and reaction temperature

Time of heating (min)	Temperature (°C)	pH	Figure No.
20	80	4.54	2
40	42	5.02	3
70	42	5.02	4

3.3.5. Stability studies

Stability experiments of the radiolabelled peptide was determined both under the storage conditions of the sample alone at 4° C (Figures 5-9) or in the environment of competitive ligand DTPA as follows: to 50 µl of radiolabelled peptide 50 µl of 10^{-3} M EDTA or DTPA solution pH 5 was added and the mixture was kept at 4°C. Concentration of the competitive ligand exceeded chelator concentration in the peptide approximately 40 times. At selected time intervals HPLC analysis of these solutions was carried out to determine radiochemical purity of peptides studied.

4. Results and discussions

The estimated purity of DOTA-Lys3-bombesin determined by HPLC analysis with UV detection is presented in Figure 1 in the Annex. The diagram shows both the main peak of the nonlabeled peptide and minor peaks corresponding to present impurities which may be residues of the reactants in the synthesis and/or degradation products of the peptide itself. These contaminants does not necessarily affect the quality of the labeled product, for example, if not free chelator is present or a shorter fragment of the peptide with preserved chelator, which may have different biological properties than the original peptide. These forms should be after radiolabelling of the sample found in the HPLC analysis as other peaks in the record.

On Figure 2 showing the results of analysis of ^{177}Lu-DOTA-Lys3-bombesin prepared at 80° C is seen on both ILLC-SG chromatograms a) and b) probable presence of multiple forms of the labeled substance even if the concentration of free radionuclide is not too high. This fact is also confirmed by HPLC analysis of that sample, where it is seen that after 20 minutes at 80° C most of the peptide was decomposed. It is clear that reaction conditions proposed for radiolabeling of minigastrins are not suitable for our peptide. Temperature is too high and reaction time is not sufficient for effective binding of radiometal to chelator.

From the comparison of HPLC analysis and thin layer chromatography (Figure 2) is clear that significantly more precise quality control provides HPLC analysis, where it is possible to determine the concentration of free radiometal besides various forms of the present fragments or otherwise changed forms of the radiolabeled peptide (It's clear also that only radiolabeled fragments are detected in this analysis with radiodetection).

Based on these initial experiments we slightly increased pH (5.02), decreased reaction temperature to 42 °C (temperature limit for radiolabeling of sensitive structures such as monoclonal antibodies) and prolonged reaction time as shown in Table 2.

Figure 3 describes quality control of ^{177}Lu-DOTA-Lys3-bombesin prepared at pH 5.02 and heated at 42° C for 40 minutes. The record shows only one peak corresponding to the labeled peptide and a very small peak between 3. – 4. minute corresponded to three percent of free radiometal in the sample. If the heating is prolonged to 70 minutes the free form of the radionuclide is not determinable but in diagram are visible two small peaks of other forms of radioactivity apparently corresponding to decomposition products of the labeled peptide (Figure 4).

Figures 5-9 are the results of further radiolabeling of ^{177}Lu-DOTA-Lys3-bombesin under similar conditions and the stability of the preparation when stored in the refrigerator for up to 28 hours from its radiolabeling. It is seen that the preparation retained its composition for at least four hours after preparation, it is also obvious that free radionuclide does not release from the bond to chelator (peak at time 3-4 minutes is not found even in the longest time of 28 hours after preparation).

The following figures (10 – 13) illustrate the results of radiolabelling of ^{177}Lu-DOTA-Lys3-bombesin after four months of storage of cold (non-radiolabeled) lyophilized peptide in a freezer at -20° C. The terms of this peptide preservation seemed to be not appropriate as the penicillin bottle closed only with a rubber stopper obviously does not protect the content enough against moisture, which the substance probably gradually adsorbed from the atmosphere.

On Figure 10 is presented quality control of ^{177}Lu-DOTA-Lys3-bombesin prepared in October 2012 (about four months saved at the conditions described above); labeling at pH 5,02, heating at 42°C lasted 40 minutes. Its visible the radiochemical purity of the preparation is worse. The chromatogram shows significant peaks of fragments of the peptide studied and the unbound radio-metal. The extension of the heating period to 70 minutes resulted in a significant degradation of the peptide (Figure 11).

On Figures 12 and 13 are presented selected results of ^{177}Lu-DOTA-Lys3-bombesin stability testing in the presence of competing ligands, EDTA or DTPA. In these experiments concentration of competing chelator exceeded concentration of DOTA bound to the peptide about 40 times. It is visible that, mainly the decomposition of the peptide took place, but also slightly increased the peak of low-molecular weight form from 3-4% of the free radio-metal to about 7%. Yet these two Figures show a good stability of the Lu-DOTA complex.

5. Annex

Figure 1: Quality control of the cold DOTA-Lys3-Bombesin carried out by HPLC analysis

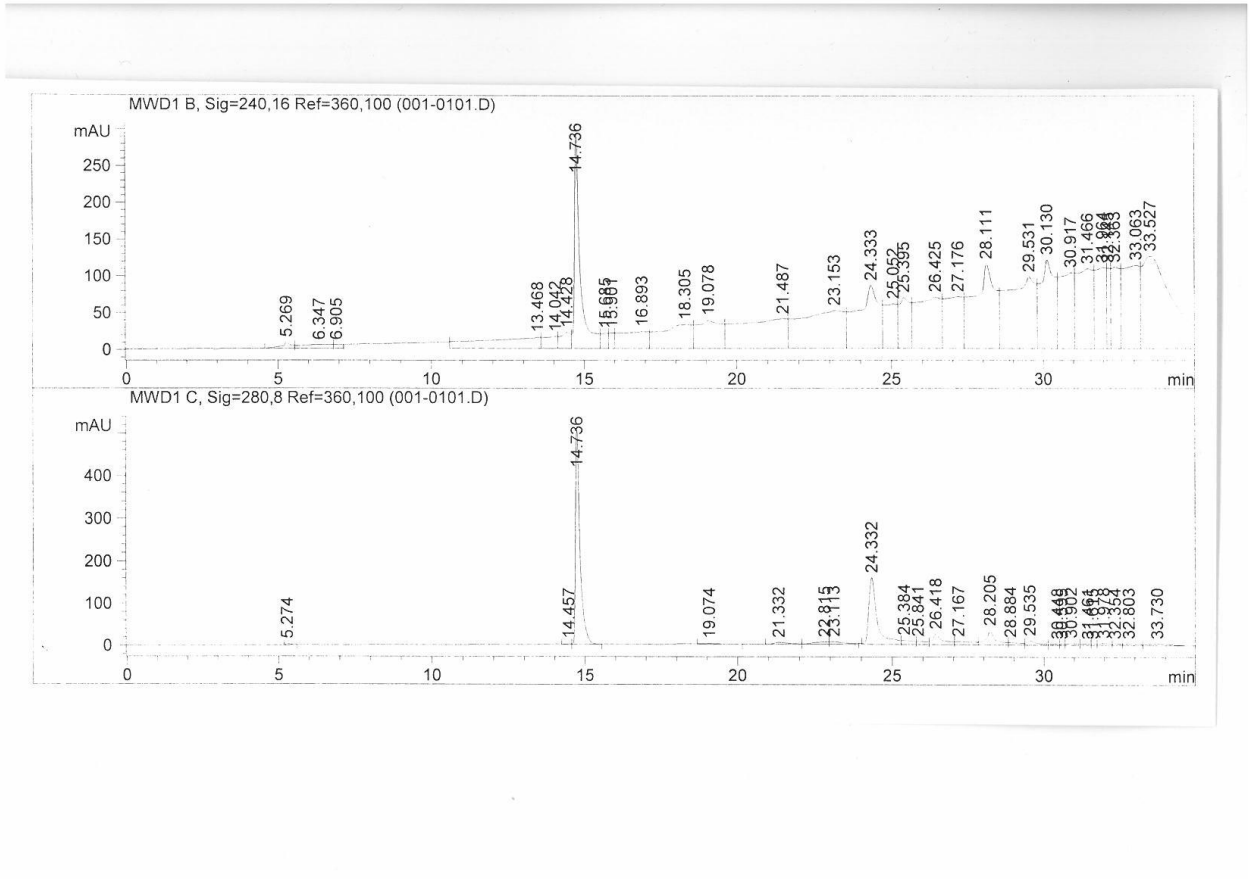


Figure 2: Quality control of ^{177}Lu-DOTA-Lys3-Bombesin prepared at 80°C, pH 4.54, heating time 20 minutes:

a) Thin layer chromatography at 10% (w/v) ammonium hydroxide water solution with methanol 1:1 (v:v),
b) Thin layer chromatography at 0.1 M ammonium acetate water solution with 10 mM EDTA pH 5.5
c) HPLC analysis

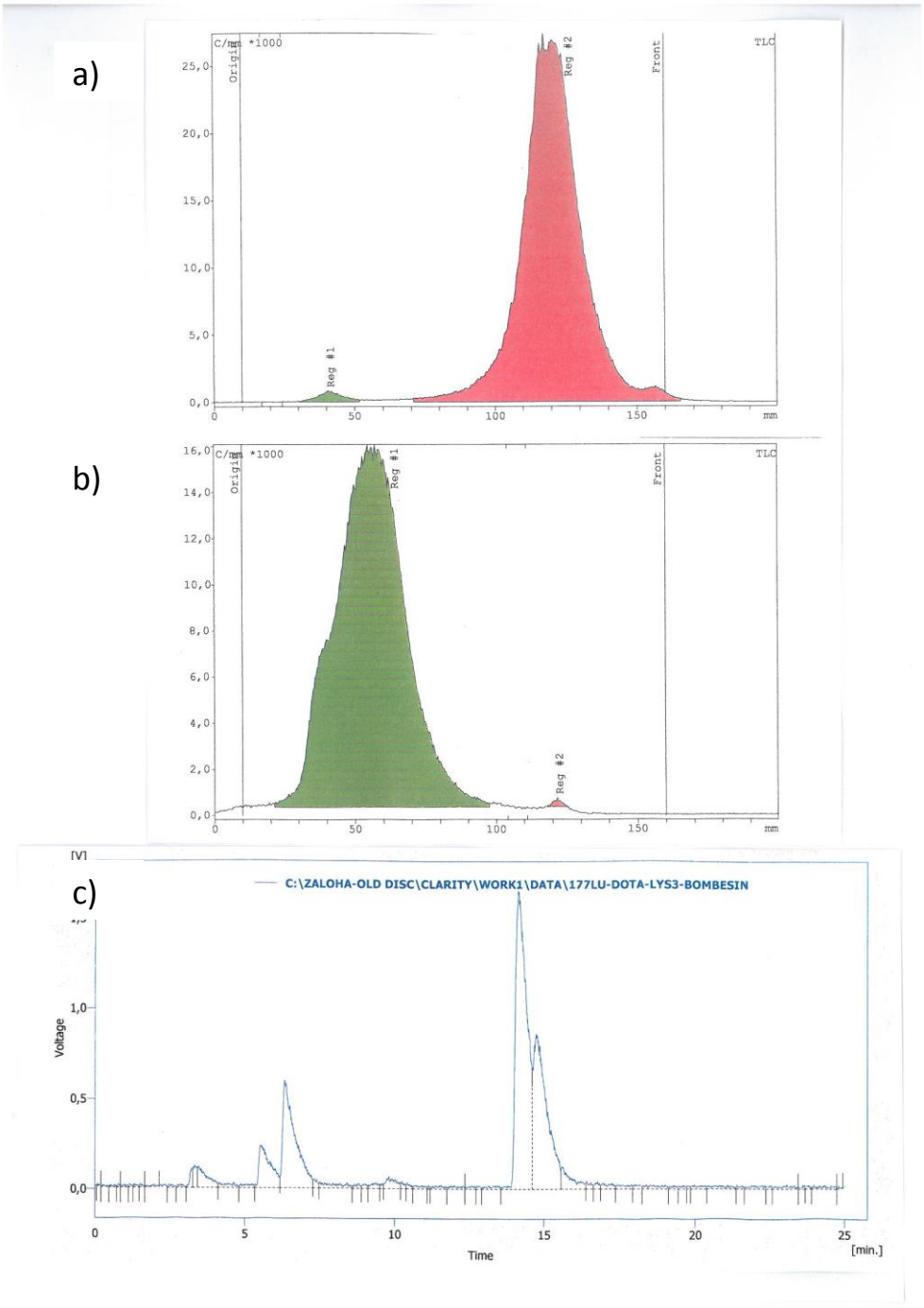
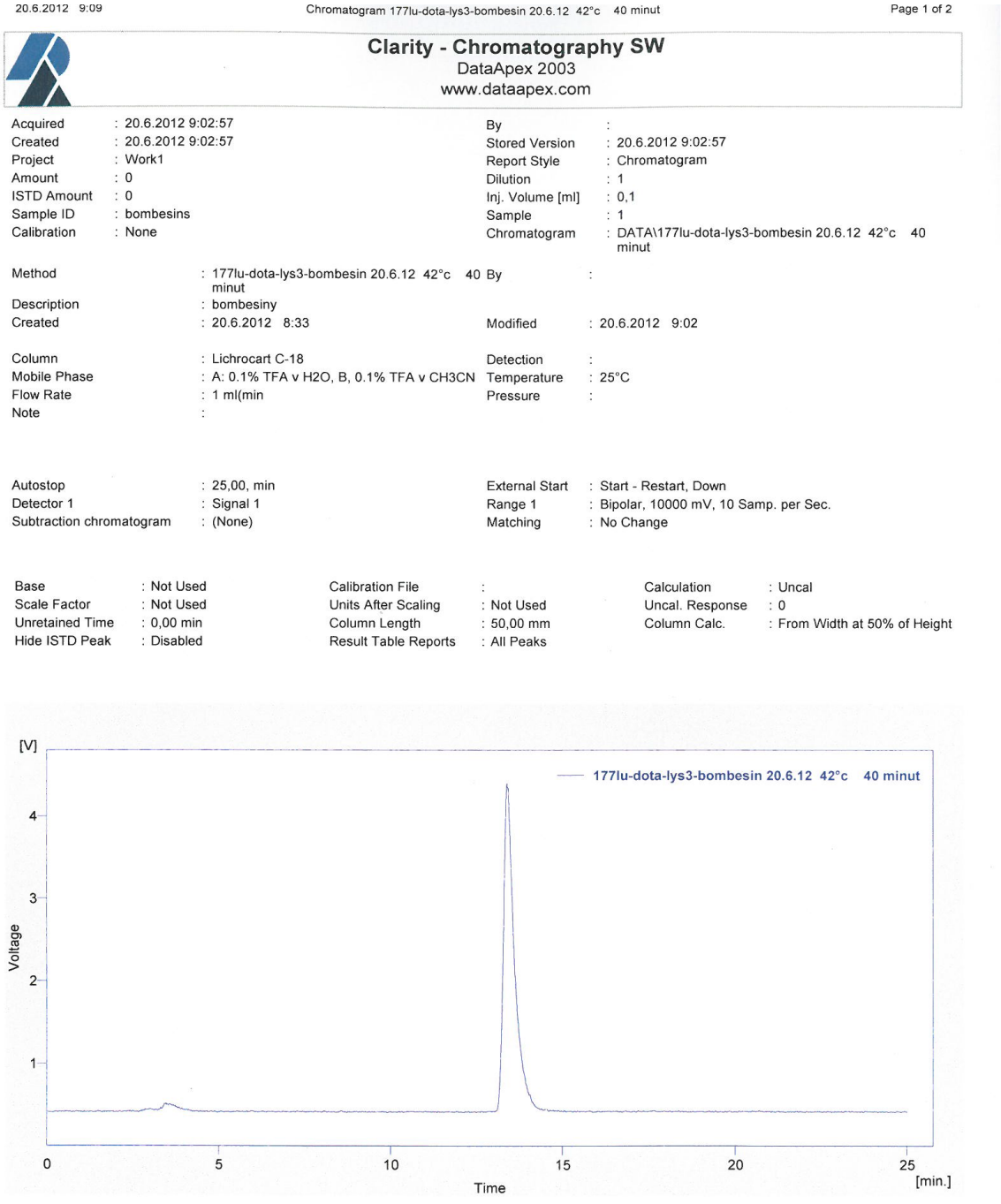


Figure 3: HPLC analysis of ^{177}Lu-DOTA-Lys3-Bombesin prepared at pH 5.02, 42°C, and heating time 40 minutes



20.6.2012 9:09 Chromatogram 177lu-dota-lys3-bombesin 20.6.12 42°c 40 minut Page 1 of 2

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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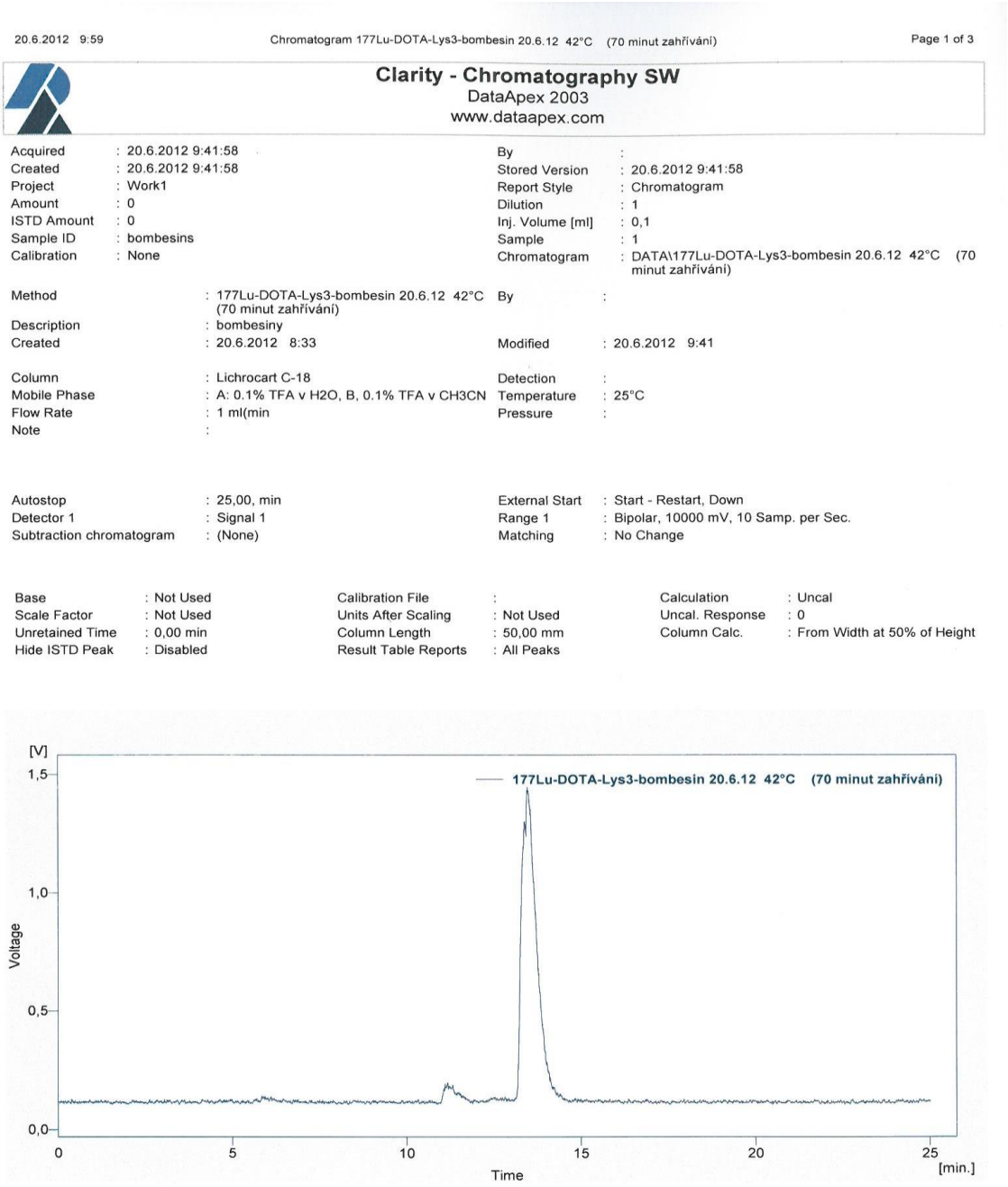
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177lu-dota-lys3-bombesin 20.6.12 42°c 40 minut

Method	: 177lu-dota-lys3-bombesin 20.6.12 42°c 40 minut	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 20.6.2012 9:02
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

Figure 4: HPLC analysis of ^{177}Lu-DOTA-Lys3-Bombesin prepared at pH 5.02, 42°C, and heating time prolonged to 70 minutes



20.6.2012 9:59 Chromatogram 177Lu-DOTA-Lys3-bombesin 20.6.12 42°C (70 minut zahřívání) Page 1 of 3

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin 20.6.12 42°C (70 minut zahřívání)

Method	: 177Lu-DOTA-Lys3-bombesin 20.6.12 42°C (70 minut zahřívání)	By	:
Description	: bombesiny		
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Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

Figure 5: Quality control of of ^{177}Lu-DOTA-Lys3-Bombesin – preparation II, the same labeling conditions (pH 5.02, 42°C, and heating time 35 minutes), HPLC analysis just after radiolabeling

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Project	: Work1	Report Style	: Chromatogram
Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177lu-dota-lys3-bombesin ii 21.6.12 42°c (35 min)

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 21.6.2012 9:20
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
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Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

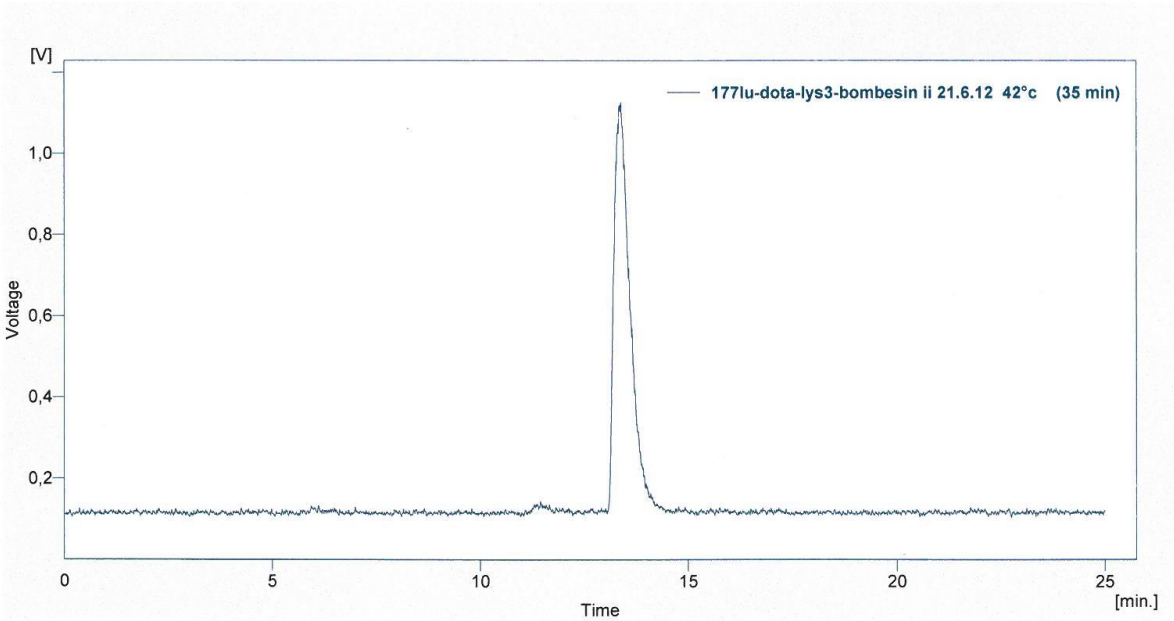

Figure 6: Quality control of of ^{177}Lu-DOTA-Lys3-Bombesin – preparation II, the same labeling conditions (pH 5.02, 42°C, and heating time 35 minutes), HPLC analysis 2 hours after radiolabeling

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177lu-dota-lys3-bombesin ii 21.6.12 (2 hod)

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 21.6.2012 10:47
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

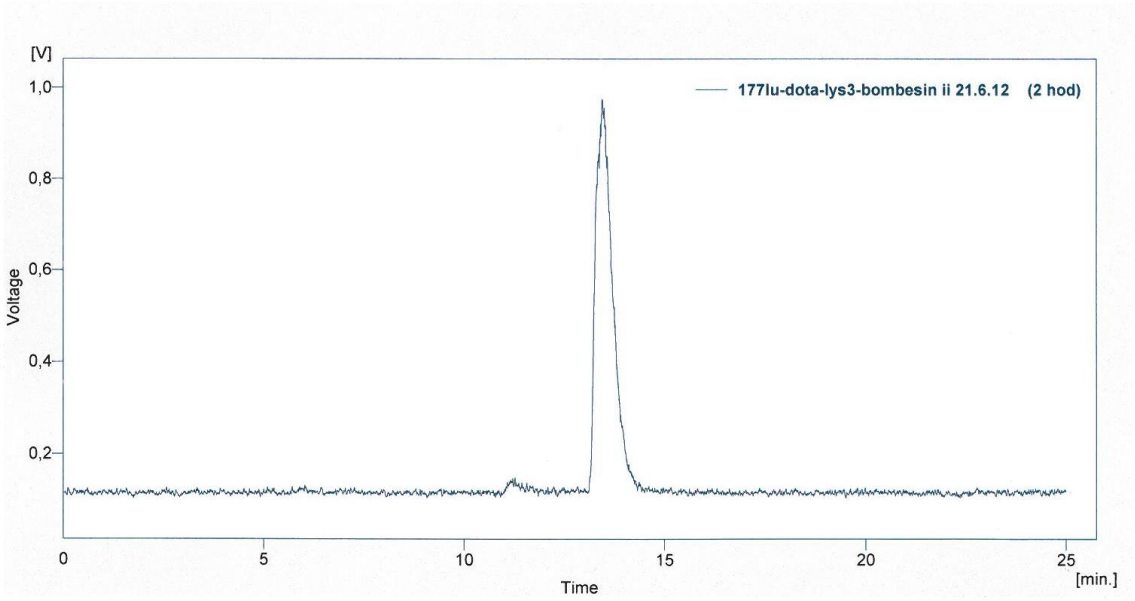

Figure 7: Quality control of of ^{177}Lu-DOTA-Lys3-Bombesin – preparation II, the same labeling conditions (pH 5.02, 42°C, and heating time 35 minutes), HPLC analysis 4 hours after radiolabeling

Clarity - Chromatography SW
DataApex 2003
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Acquired	: 21.6.2012 12:45:09	By	:
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Project	: Work1	Report Style	: Chromatogram
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ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177lu-dota-lys3-bombesin ii 21.6.12 (4 hod)

Method	: Bombesiny	By	:
Description	: bombesiny		
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Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
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Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

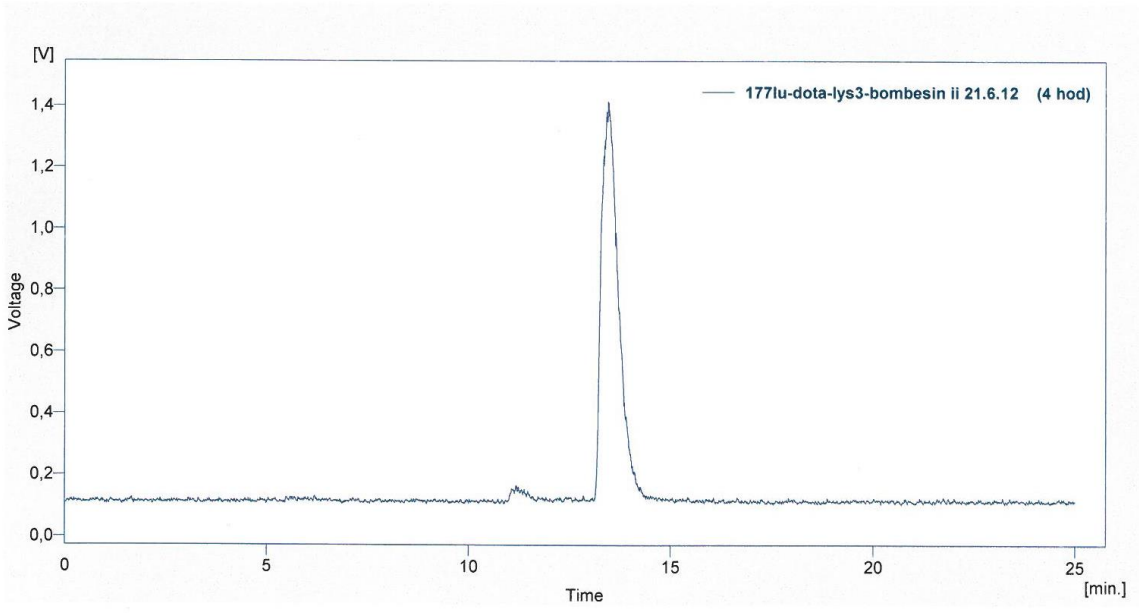
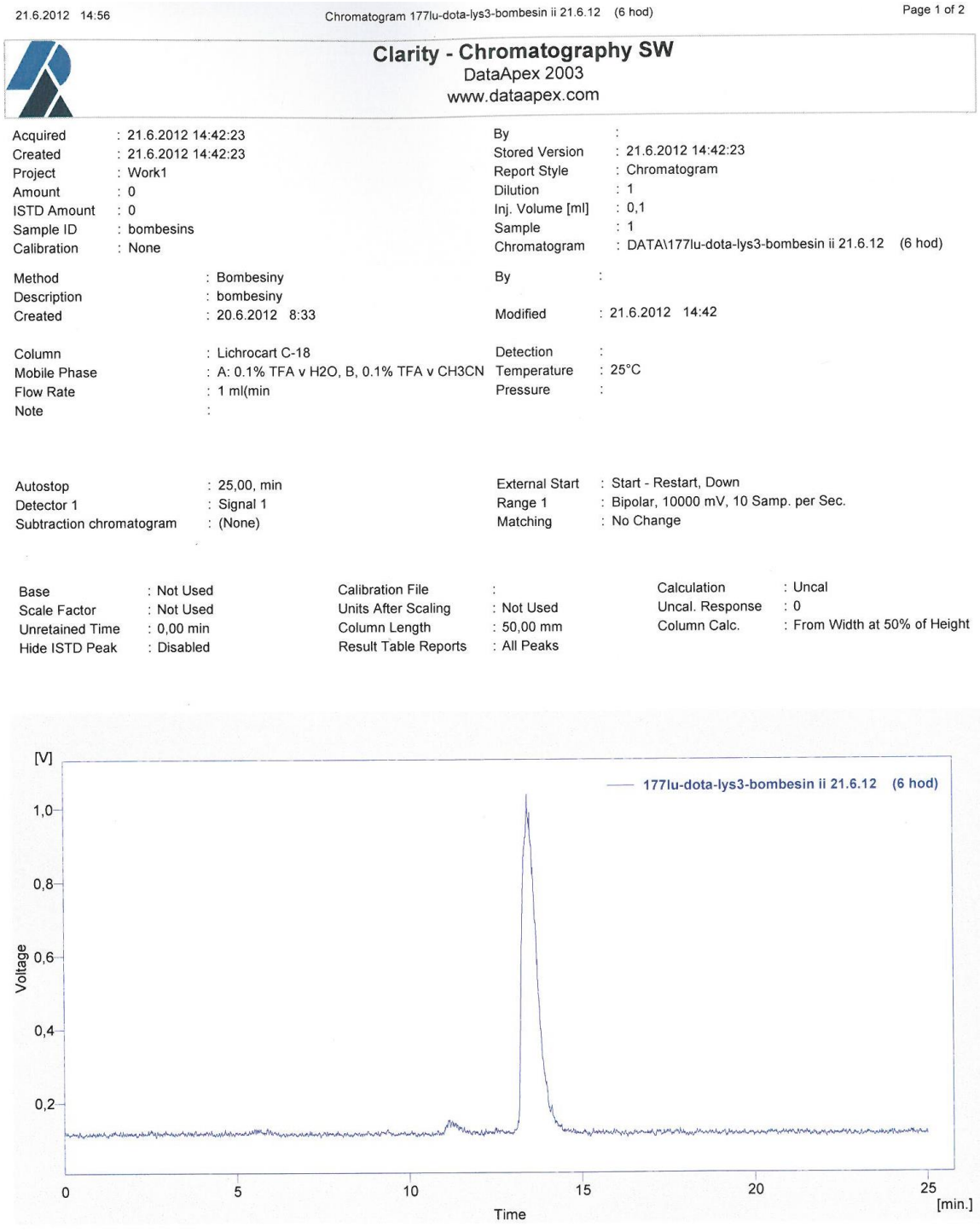


Figure 8: Quality control of of ^{177}Lu-DOTA-Lys3-Bombesin – preparation II, the same labeling conditions (pH 5.02, 42°C, and heating time 35 minutes), HPLC analysis 6 hours after radiolabeling



21.6.2012 14:56 Chromatogram 177lu-dota-lys3-bombesin ii 21.6.12 (6 hod) Page 1 of 2

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Project	: Work1	Report Style	: Chromatogram
Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177lu-dota-lys3-bombesin ii 21.6.12 (6 hod)

Method	: Bombesiny	By	:
Description	: bombesiny		
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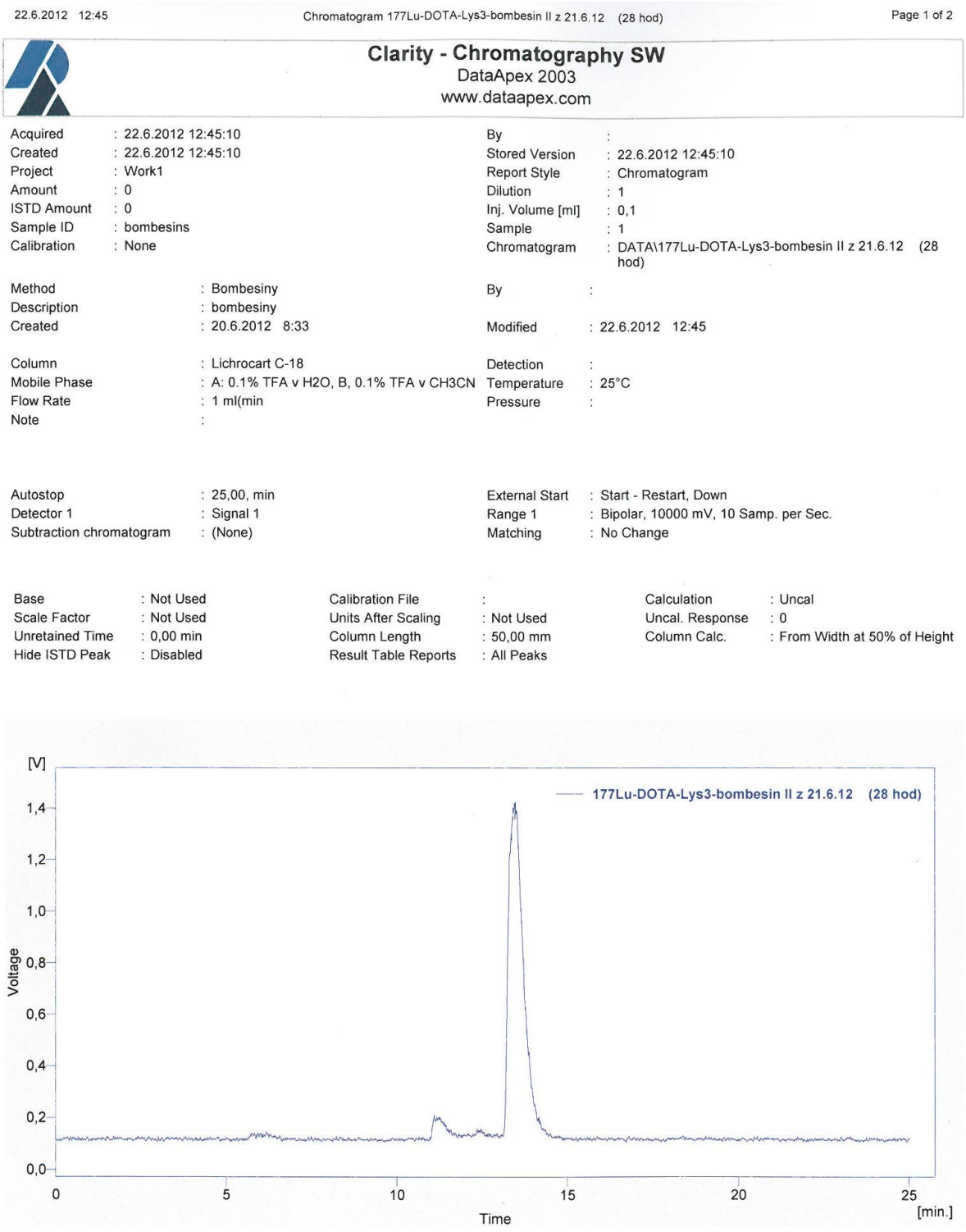
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

Figure 9: Quality control of of ^{177}Lu-DOTA-Lys3-Bombesin – preparation II, the same labeling conditions (pH 5.02, 42°C, and heating time 35 minutes), HPLC analysis 28 hours after radiolabeling



22.6.2012 12:45 Chromatogram 177Lu-DOTA-Lys3-bombesin II z 21.6.12 (28 hod) Page 1 of 2

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Created	: 22.6.2012 12:45:10	Stored Version	: 22.6.2012 12:45:10
Project	: Work1	Report Style	: Chromatogram
Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin II z 21.6.12 (28 hod)

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 22.6.2012 12:45
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

Figure 10: Quality control of ^{177}Lu-DOTA-Lys3-Bombesin prepared 4 months after preservation of the cold peptide at -20°C, labeling conditions (pH 5.02, 42°C, and heating time 40 minutes), HPLC analysis 15 minutes after radiolabeling

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin II 17.10.12

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 17.10.2012 15:53
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN (New B)	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

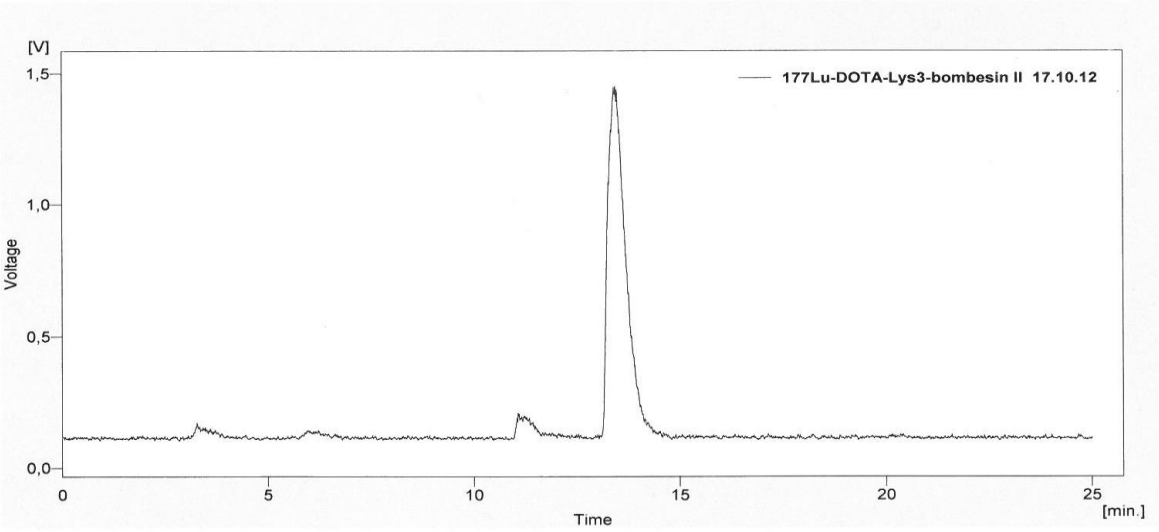

Figure 11: Quality control of ^{177}Lu-DOTA-Lys3-Bombesin prepared 4 months after preservation of the cold peptide at -20°C, labeling conditions (pH 5.02, 42°C, and heating time 70 minutes), HPLC analysis 15 minutes after radiolabeling

17.10.2012 11:36 Chromatogram 177Lu-DOTA-Lys3-bombesin 17.10.12 70 min Page 1 of 2

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

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Created	: 17.10.2012 11:33:31	Stored Version	: 17.10.2012 11:33:31
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ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin 17.10.12 70 min

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 17.10.2012 11:33
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

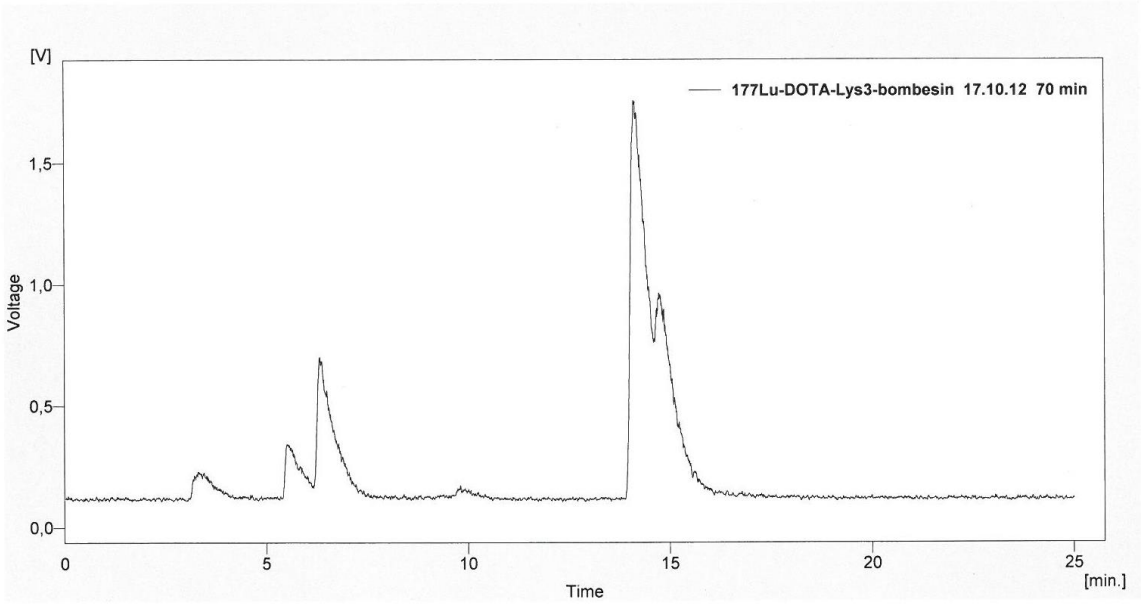


Figure 12: Quality control of ^{177}Lu-DOTA-Lys3-Bombesin after 28 hours incubation in the competitive chelator EDTA (quality of original peptide was presented on Figure 11)

Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

Acquired	: 18.10.2012 19:15:39	By	:
Created	: 18.10.2012 19:15:39	Stored Version	: 18.10.2012 19:15:39
Project	: Work1	Report Style	: Chromatogram
Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin II. z 17.10.12 v EDTA 24h

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 18.10.2012 19:15
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN (New B)	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		

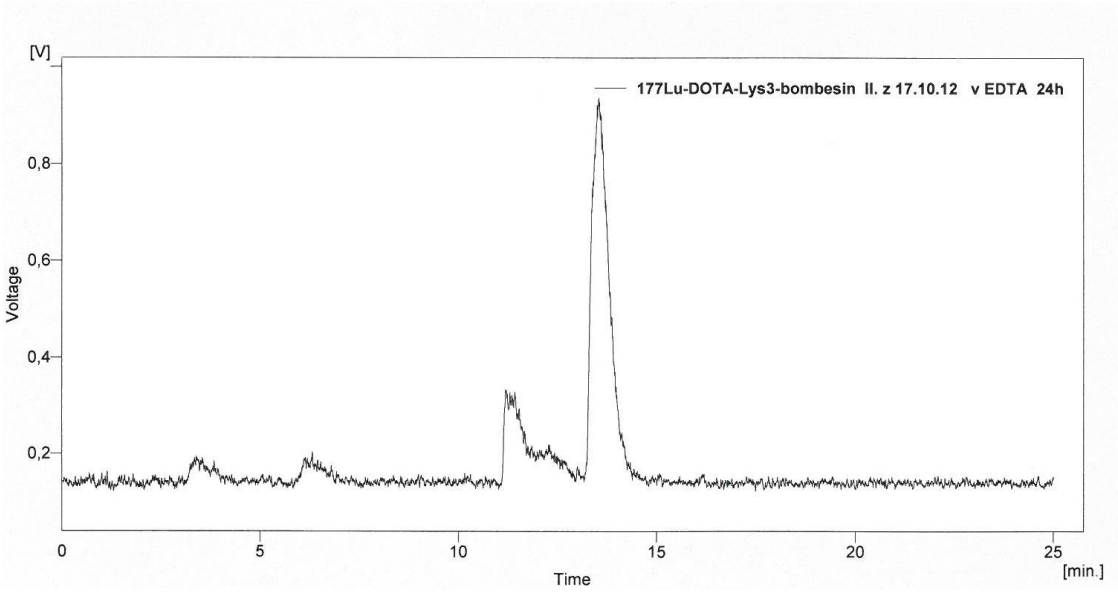


Figure 13: Quality control of ^{177}Lu-DOTA-Lys3-Bombesin after 28 hours incubation in the competitive chelator DTPA (quality of original peptide was presented on Figure 10)

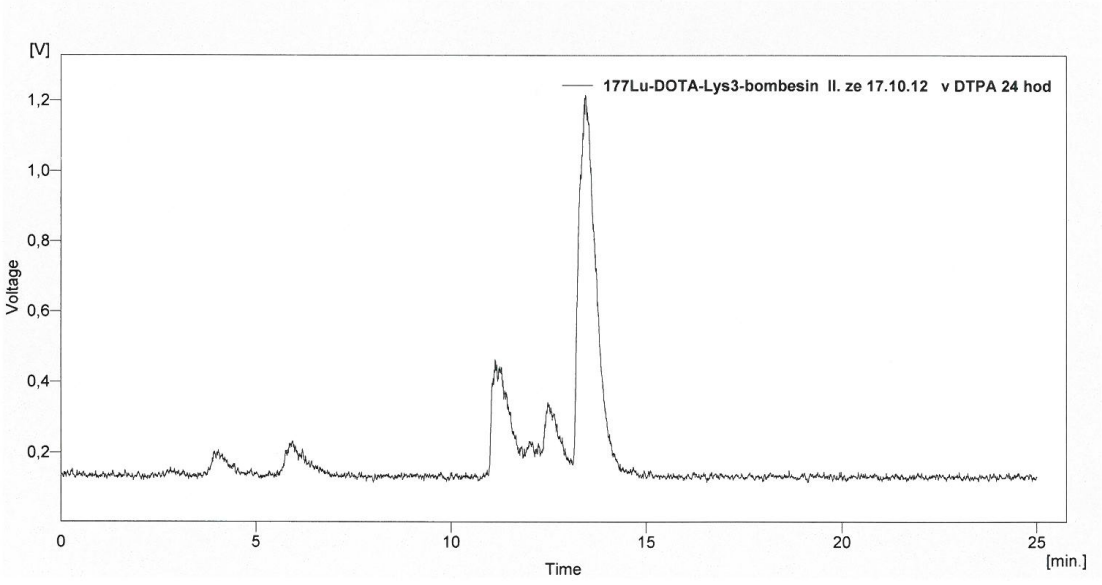
Clarity - Chromatography SW
DataApex 2003
www.dataapex.com

Acquired	: 19.10.2012 11:29:44	By	:
Created	: 19.10.2012 11:29:44	Stored Version	: 19.10.2012 11:29:44
Project	: Work1	Report Style	: Chromatogram
Amount	: 0	Dilution	: 1
ISTD Amount	: 0	Inj. Volume [ml]	: 0,1
Sample ID	: bombesins	Sample	: 1
Calibration	: None	Chromatogram	: DATA\177Lu-DOTA-Lys3-bombesin II. ze 17.10.12 v DTPA 24 hod

Method	: Bombesiny	By	:
Description	: bombesiny		
Created	: 20.6.2012 8:33	Modified	: 19.10.2012 11:29
Column	: Lichrocart C-18	Detection	:
Mobile Phase	: A: 0.1% TFA v H2O, B, 0.1% TFA v CH3CN (New B)	Temperature	: 25°C
Flow Rate	: 1 ml(min	Pressure	:
Note	:		

Autostop	: 25,00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 10000 mV, 10 Samp. per Sec.
Subtraction chromatogram	: (None)	Matching	: No Change

Base	: Not Used	Calibration File	:	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Unretained Time	: 0,00 min	Column Length	: 50,00 mm	Column Calc.	: From Width at 50% of Height
Hide ISTD Peak	: Disabled	Result Table Reports	: All Peaks		



6. Conclusions

1. In the framework of this thesis a study of radiolabeling of DOTA- Lys3-Bombesin with therapeutic radionuclide ^{177}Lu was conducted.

2. Conditions for the labeling of the peptide studied with this radionuclide were found in the quality required for biodistribution studies (purity greater than 97%).

3. Comparison of methods for determination of radiochemical purity of the radiolabeled peptide showed greater reliability of HPLC analysis compared to thin-layer chromatography.

4. The labeled peptide was stable at 4° C for at least four hours from its preparation; stability studies in the environment of competing ligand showed a good stability of the chelate DOTA with Lu.

7. List of Abbreviations

BBN	Bombesin
GRP-R	Gastrin Releasing Peptide-Receptor
NMB-R	Neuromedin B –Receptor
PET	Positron Emission Tomography
SPECT	Single photon Emission Computed Tomography
BFC	Bifunctional Chelate
EDTA	Diethylenetriaminepentaacetic acid
DTPA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
NOTA	1,4,7-triazacyclononane-N,N',N''-triacetic acid
TETA	1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid
HYNIC	Hydrazino nicotinic acid
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography

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