

Optimized small scale automated preparation and quality control of [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 for clinical application

Sven Messerschmidt

Medical University of Innsbruck: Medizinische Universität Innsbruck

Taraneh Sadat Zavvar

Medical University of Innsbruck: Medizinische Universität Innsbruck

Lisa Pivetta

Medical University of Innsbruck: Medizinische Universität Innsbruck

Martin Kraihammer

Medical University of Innsbruck: Medizinische Universität Innsbruck

Klaus Faserl

Medical University of Innsbruck: Medizinische Universität Innsbruck

Paul Eichberger

Medical University of Innsbruck: Medizinische Universität Innsbruck

Clemens Decristoforo

Medical University of Innsbruck: Medizinische Universität Innsbruck

Elisabeth von Guggenberg

Medical University of Innsbruck: Medizinische Universität Innsbruck

Emanuel Sporer

emanuel.sporer@i-med.ac.at

Medical University of Innsbruck: Medizinische Universität Innsbruck <https://orcid.org/0009-0006-2514-255X>

Research Article

Keywords: cholecystokinin-2 receptor, minigastrin, gallium-68, lutetium-177, radiopharmacy, automation

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11000682/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The minigastrin analogue DOTA-MGS5 targeting the cholecystokinin-2 receptor has shown promise as a theranostic agent for medullary thyroid cancer and small cell lung cancer when labelled with gallium-68 or lutetium-177. This study validated an optimized automated synthesis protocol for routine production of these radiopharmaceuticals.

Using a modular synthesizer, a multi-step synthesis process that eliminated concentration of the ^{68}Ga -eluate and post-labelling product purification was developed. Safe acceptance criteria for the final products were established, alongside with a systemic characterization of the potential impurities, supporting the robust in house production.

For $[^{68}\text{Ga}]\text{Ga}$ -DOTA-MGS5, nine batches with activity of 710 ± 117 MBq were produced. The addition of a radical scavenger to the reaction buffer was crucial in reducing peptide-related radiolytic side products. Particularly 0.1% ascorbic acid allowed to achieve a product with > 95% purity and a shelf-life of 2h. The three batches of $[^{177}\text{Lu}]\text{Lu}$ -DOTA-MGS5 with activity of 7371 ± 1190 MBq showed a similar quality, with the product demonstrating an excellent stability with less than 3% radiolysis up to 24 h post preparation. The peptide precursor, the radiolabelled metal complexes, as well as Fe-DOTA-MGS5 and Zn-DOTA-MGS5 identified in the final product, all showed comparable receptor affinity. Radiolytic oxidative processes led to single and double oxidation of tryptophan and/or tyrosine.

The simplified automated synthesis process established for both radiopharmaceuticals mitigate radiolysis while ensuring high product quality. By avoiding complex purification steps, the optimized workflow supports a more efficient and reliable production process suitable for routine clinical application.

Introduction

DOTA-MGS5, a minigastrin (MG) analogue targeting the cholecystokinin-2 receptor (CCK2R), has emerged as a promising theranostic agent for both targeted radionuclide imaging and therapy [1, 2]. The ^{68}Ga - and ^{177}Lu -labelled conjugates exhibit high affinity for CCK2R, which is highly expressed in several malignancies, including medullary thyroid carcinoma (MTC, ~ 92%), small-cell lung cancer (SCLC, ~ 57%), astrocytoma (~ 65%), and ovarian stromal tumors (100%) [3–5]. In preclinical and first clinical evaluations, these new radiopharmaceuticals have demonstrated high stability and favourable pharmacokinetics, supporting their further clinical translation and evaluation [5–9].

We have recently reported on the automated, cassette-based radiosynthesis of $[^{68}\text{Ga}]\text{Ga}$ -DOTA-MGS5 [10] including a concentration and purification step of the ^{68}Ga -eluate as well as a post-labelling product purification step. Also the automated synthesis of $[^{177}\text{Lu}]\text{Lu}$ -DOTA-MGS5 was based on a multi-step cassette based workflow including a post-labelling product purification step [2]. Cassette-based workflows enhance reproducibility, reduce operator variability, improve radiation safety, and shorten production times especially when working with short-life radioisotopes [11, 12]. Moreover process automation allows for standardized handling and closed operation allowing to meet the regulatory requirements for a GMP-compliant production [13–16]. Special attention has to be paid to the choice of reagents and single-use materials to minimize the risk of chemical and microbiological contamination. In the case of radiometal-based radiopharmaceuticals, the presence of metal ion impurities needs to be reduced to a minimum, to avoid that other metal ions compete with the radiometal in the complexation reaction, resulting in non-quantitative incorporation of the radiometal. The high radioactivity concentrations used

in the synthesis process, increase the risk of radiolysis caused due to the formation of free radicals during the reaction. We could recently show, that solid phase extraction (SPE) used as a final product purification step for the separation of the free radiometal as well as radiocolloids from the final formulation has potential increase radiolytic impurities in the final product [17, 18].

During synthesis validation and product formulation these aspects need to be thoroughly addressed to establish safe acceptance criteria for the final product. Comprehensive impurity profiling includes both chemical and radiochemical impurities. Within the frameworks of the International Council for Harmonisation (ICH), European Pharmacopoeia (Ph. Eur.) and United States Pharmacopeia (USP) specific guidance and legally binding standards for impurity testing are given. For radiopharmaceuticals, this mandate spans radionuclidic purity, radiochemical purity, chemical purity, residual solvents, and sterility. Radiochemical purity testing includes the assessment of all radioactive species present in the final product. Non-radioactive testing includes the determination the determination of the non-radioactive peptide precursor and related substances in the final formulation. In addition, in-process controls are being carried out to monitor the automated process.

In the present work an optimized, purification-free synthetic protocol for [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 has been validated. The new processes are designed to mitigate radiolysis while maintaining product quality, thereby facilitating a more robust routine production. Furthermore a systematic characterization of potential impurities has been carried out for both radiopharmaceuticals.

Methods

Materials and reagents

A pharmaceutical grade $^{68}\text{Ge}/^{68}\text{Ga}$ generator with a nominal activity of 1850 MBq and eluted with 0.1 N hydrochloric acid was purchased from Eckert & Ziegler (Berlin, Germany). Pharmaceutical grade [^{177}Lu]LuCl₃ (non-carrier added) in 0.04 N hydrochloric acid (HCl) was purchased from different commercial suppliers (ITM Medical Isotopes, Garching/Munich, Germany or Isotopia Nuclear Medicine, Park Tetach Tikva, Isreal). The chemical precursor DOTA-MGS5 in GMP quality was obtained from piCHEM (Raaba Grambach, Austria) in aliquots of 250 µg. Pharmaceutical grade products or ultra-pure reagents were used for the radiolabelling process. For the dissolution of the peptide precursor 95% ethanol (B. Braun Melsungen AG, Melsungen, Germany) and aqua bidestillata (Fresenius Kabi Austria GmbH, Graz, Austria) were used. The reaction buffers for the radiolabelling process were prepared using sodium acetate trihydrate (prod.n. 1.06267; Merck, Darmstadt, Germany), ascorbic acid (prod.n. 95210, Sigma-Aldrich St. Louis, MO, USA), sodium hydroxide monohydrate (prod.n. 1.06466; Merck, Darmstadt, Germany), 30% hydrochloric acid (prod.n. 1.01514, Merck, Darmstadt, Germany) and ultrapure water (prod.n. 14211; Sigma-Aldrich St. Louis, MO, USA). Calcium trisodium pentetate (Ditripentat-Heyl®, Heyl, Berlin, Germany), ascorbic acid (Pascoe, Giessen, Germany), and physiological saline (Fresenius Kabi, Graz, Austria) were used for the formulation of the final product. The use of 1 M sodium bicarbonate (Fresenius Kabi, Graz, Austria) for pH adjustment of the final preparation was also tested. All other reagents obtained from commercial suppliers were of analytical grade.

Synthesis Module

For the labelling process, a modular cassette-based synthesizer (Modular-Lab PharmTracer, Eckert & Ziegler, Berlin, Germany) consisting of a syringe module, a heater reaction module and a 2-fold module was used. All steps of the process were defined and tracked using the equipment-specific software (Modular-Lab SoftPLC 6.2). An additional 1-fold module was used to perform a cassette pressure test with nitrogen before starting the process to ensure the integrity of the cassette as well as a sterile filter pressure hold test after completing the radiosynthesis. A pre-assembled, sterilised cassette consisting of two 3 stopcocks manifolds and with a customized layout was used for the synthesis process. Before starting the process, the cassette is mounted into the module, the gas line connected to ventilation filter, the reaction vial placed in the heater, the waste line connected to the waste, and the product line connected to the product vial through a sterile filter. All reagents and single-use materials needed for the radiolabelling process were prepared and assembled in the laminar air flow.

Radiolabelling process with gallium-68

For the preparation of the reaction buffer 1.50 g sodium acetate trihydrate and 10 ml of ultrapure water were added to a 50 mL Falcon tube and the solution pH adjusted to 5.5 using a 30% hydrochloric acid (~ 150 µL). Aliquots of 1 mL were sterile filtered and stored in cryotubes at -20°C. For the dissolution of DOTA-MGS5, the crimp cap and rubber of the vial with 250 µg DOTA-MGS5 was removed and the lyophilisate dissolved with 50 µL 95% ethanol and 200 µL aqua bidestillata, using sterile pipette tips. A 50 µL aliquot of this solution was added to the reaction buffer.

To assess the effect of radio-protective scavengers the addition of 200 µL 95% ethanol and 100 µL ascorbic acid solution (50 mg/mL) to the reactor were tested. Three syntheses were performed for each reaction condition used.

Before starting the synthesis process the generator line was connected to the cassette, the 50 ml vial of physiological saline fixed on the spike of the cassette, and the reaction buffer containing the peptide and the tested radio-protective scavenger transferred into the reactor using a syringe. The synthesis scheme for the radiolabelling process is shown in Fig. 1A.

The radiolabelling process begins with the elution of the $^{68}\text{Ge}/^{68}\text{Ga}$ generator with direct transfer of the eluate (5 ± 1 mL) containing gallium-68 in 0.1 M hydrochloric acid ($[^{68}\text{Ga}]\text{GaCl}_3$) into the reactor, followed by a heating step at 95°C for 7 min. After completion of the heating step, all lines are flushed with physiological saline, followed by the transfer of the reaction solution to the product vial through the sterile filter, and dilution with physiological saline to a final volume of ≤ 15 mL. The process also includes a designated vial for possible pH adjustment of the final formulation with 1 mL 1 M sodium bicarbonate.

Radiolabelling process with lutetium-177

For the preparation of the reaction buffer 0.55 g ascorbic acid and 10 ml of ultrapure water were added to a 50 mL Falcon tube and the solution was pH adjusted to 4.5 using a 30% sodium hydroxide solution (~ 0.5 mL). Aliquots of 1.0 mL were sterile filtered and stored at -20°C in cryotubes. A 3 mg/mL DTPA solution was prepared diluting calcium trisodium pentetate with physiological saline. Using a 0.22 µm sterile filter (Millex GV, SLGV013SL, Merck Millipore, Co. Cork, Ireland), 0.3 mL of the DTPA solution and 1 mL of ascorbic acid solution (150 mg/mL) were added to the product vial to stabilize the final formulation. For the dissolution of DOTA-MGS5, the crimp cap and

rubber of the vial with 250 µg DOTA-MGS5 was removed and the lyophilisate dissolved with 50 µL 95% ethanol and 200 µL aqua bidestillata, using sterile pipette tips. A 100 µL aliquot and 200 µL of aqua bidestillata were transferred into a cryotube, using sterile pipette tips.

Before starting the synthesis process the vial containing Lutetium-177 in 0.04 N hydrochloric acid ($[^{177}\text{Lu}]\text{LuCl}_3$) was connected to the activity line, a 50 ml vial of physiological saline fixed on the spike of the cassette, the reaction buffer transferred in the designated vial of the cassette and the peptide solution transferred into the reactor using a syringe. The synthesis scheme for the radiolabelling process is shown in Fig. 1B.

The radiolabelling process begins with the transfer of the ^{177}Lu -chloride solution into the reactor, followed by the transfer of the reaction buffer first to the ^{177}Lu -chloride vial and then to the reactor. After a heating step at 90°C for 20, all lines are flushed with physiological saline, followed by the transfer of the reaction solution to the product vial through the sterile filter and dilution with physiological saline to a final volume of ≤ 15 mL.

Quality control

Radioactivity measurements were performed using a VDC-405 dose calibrator (Veenstra Instruments, Joure, Netherlands). Identification of the radionuclide was carried out by gamma ray spectrometry (GabiStar, Raytest, Straubenhardt, Germany) and half-life calculation.

For HPLC analysis, two UltiMate 3000 chromatography systems (Dionex, Germering, Germany) were used. The systems are composed of a HPLC pump, an auto-sampler, a column compartment allowing for a controlled temperature of the column of 25°C, a variable wavelength detector and a γ -detector (Gabi, Raytest, Straubenhardt, Germany). In HPLC method 1 – used for quality control – the variable wave length detector was set at 280 nm and an ACE C18 column (150 × 3 mm, 3 µm ACE-111-1503, Avantor Sciences, Pennsylvania, United States of America) was used with the following gradient of water and acetonitrile (ACN) containing 0.1% trifluoroacetic (TFA) acid and flow-rate of 0.6 mL/min: 0–2 min 28% ACN, 2–12 min 28–55% ACN, 12–12.1 min 55 – 28% ACN, 12.1–16 min 28% ACN. In HPLC method 2 – used for characterization – the variable wavelength detector was set at 220 nm and a Nucleodur C18 HTec (250 x 4.6 mm, 5 µm, REF 760316.46, Macherey-Nagel, Düren, Germany) column with a Nucleodur C18 HTec (4 x 3 mm, 5 µm, REF 718966, Macherey-Nagel, Düren, Germany) pre-column was used with the following gradient of water and acetonitrile (ACN) containing 0.1% TFA at a flow-rate of 1.0 mL/min: 0–1 min 27% ACN, 1–30 min 27–40% ACN, 30–30.1 min 40–80% ACN, 30.1–31 min 80% ACN, 31–31.1 min 80 – 27% ACN, 31.1–35 min 27% ACN. For the testing of DOTA-MGS5, Ga-DOTA-MGS5/Lu-DOTA-MGS5 and other related substances, reference solutions with a concentration of 0.05–0.1 g/L were used. The peptide amount in the final product was determined from the UV chromatogram based on a calibration curve generated with five dilutions of DOTA-MGS5 in the range of 1–100 µg/mL. For the determination of the reporting threshold in the UV chromatogram, a reference solution with 50 µg DOTA-MGS5 in 15 ml was prepared. An aliquot of 1 mL of this reference solution was diluted to 10 mL (1:10, v:v) and subjected to HPLC analysis to define the disregard limit for the maximum administered volume of 15 mL corresponding to a peptide content < 5 µg in the entire product. UV peaks below this limit were not reported and not further characterized. In addition to DOTA-MGS5, Ga-DOTA-MGS5, Lu-DOTA-MGS5, also other metal complexes of DOTA-MGS5 were analysed using HPLC method 1 and 2 (Fe-DOTA-MGS5, Zn-DOTA-MGS5, Cu-DOTA-MGS5, Ni-DOTA-MGS5) to identify the other related substances in the UV-chromatogram. Different metal complexes were prepared using $\text{Ga}(\text{NO}_3)_3$ (gallium(III) nitrate), LuCl_3 (lutetium(III) chloride), FeCl_3 (iron(III) chloride), $\text{Zn}(\text{CH}_3\text{COO})_2$ (zinc(II) acetate), $\text{Ca}(\text{CH}_3\text{COO})_2$ (copper(II) acetate)

and NiCl_2 (nickel(II) chloride) in 0.05 M HCl. DOTA-MGS5 (50–100 μg) was reacted with a 3-fold excess of the respective metal using a 0.4–0.8 ammonium acetate buffer pH adjusted to pH 4–5 in a low protein binding Eppendorf tube for 15 min at 85°C. The identity of each metal-complex in the reference solutions was determined using mass spectrometry (Nexera LC/MS-2050, Shimadzu, Kyoto, Japan). For confirmation of the process related formation of metal complexes, automated syntheses performed without connecting the $^{68}\text{Ge}/^{68}\text{Ga}$ -generator or the $[^{177}\text{Lu}]\text{LuCl}_3$ vial were also subjected to HPLC and mass analysis.

In addition, Ga-DOTA-MGS5 and Lu-DOTA-MGS5, prepared using ammonium acetate buffer in a low protein binding Eppendorf tube, were subjected to x-ray irradiation to mimic radiolysis. The Eppendorf tubes with the metal complexes (50 μg , 500 μl) were irradiated in a GSR C1 gamma irradiation device (Gamma-Service Medical GmbH, Leipzig, Germany) and irradiated to total doses of 200 Gy, using an average dose rate of approximately 4.32 Gy/min. To identify the radiolysis-induced compounds present in the individual HPLC peaks, 2 μL of each fraction were loaded onto an UltiMate 3000 nano-HPLC system coupled to a Q Exactive HF mass spectrometer (Thermo Scientific). Analytes were separated on a 17cm long column (100 μm i.d.) packed with 2.4 μm C18 material (Reprosil). Separation was performed using a gradient (solvent A: 0.1% formic acid; solvent B: 0.1% formic acid in 85% acetonitrile) starting at 4% B which was increased linearly to 50% during 20 min and from 50% to 100% during 5 min. A flowrate of 250 nL/min was applied. The Q Exactive was operated in data-dependent acquisition mode with a 1 s duty cycle. Full MS scans were acquired from 375 to 1500 m/z at a resolution of 60 000. The most abundant precursor ions from the full MS scan were selected for higher-energy collisional dissociation (HCD) fragmentation using an isolation window of 1.2 m/z and a normalized collision energy of 30, with MS/MS spectra acquired at a resolution of 15,000. Raw data files were analyzed using Qual Browser software (Version 4.8, Thermo Scientific) to determine the accurate masses of precursor and fragment ions, enabling the structural characterization of radiolysis by-products.

For quality control, a 10–20 μL sample of the ^{68}Ga -labelled DOTA-MGS5 product was analysed by HPLC and data analysis was performed using the equipment-specific software (Chromeleon 7). The identity of the final product was assessed considering a relative retention time (RRT) of 9.9–1.1 in comparison with the retention time of the reference standard Ga-DOTA-MGS5 or Lu-DOTA-MGS5. The radiochemical purity was assessed by peak analysis of the principal peak due to $[^{68}\text{Ga}]\text{Ga-DOTA-MGS5}$ or $[^{177}\text{Lu}]\text{Lu-DOTA-MGS5}$ in the radiochromatogram. All peaks with RRT of 0.7–1.2 compared to the principal peak were considered as radiochemical impurities related to radiolytic side products. Peaks with RRT < 0.2 – attributed to free gallium-68/lutetium-177 – were not considered, as free gallium-68/lutetium-177 and radiocolloids were analysed by TLC.

Instant TLC (iTLC) analysis was performed using iTLC-SG glass microfiber chromatography paper impregnated with silica gel of 9 x 1 cm (Varian iTLC-SG, Agilent Technologies, California, USA) pre-treated at 110°C for 30 min and using two mobile phases: system 1 with 0.1 M sodium citrate pH 5 and system 2 with 1 M ammonium acetate/methanol (MeOH) (1:1, v/v). The radioactivity on the strips was measured using a Scan-Ram radio-TLC-scanner with a PS Plastic/PMT PS detector (LabLogic, Sheffield, UK) and data analysis was performed using the equipment-specific software (Laura Radiopharma). $[^{68}\text{Ga}]\text{Ga-DOTA-MGS5}$ and $[^{177}\text{Lu}]\text{Lu-DOTA-MGS5}$ showed a R_f of < 0.2 in system 1 and of > 0.8 in system 2. The radioactive impurities calculated from iTLC analysis account for free lutetium-177 (R_f > 0.8 in system 1) and radiocolloid (R_f < 0.2 in system 2). To assess product stability, HPLC and iTLC analysis, were repeated at different time points after preparation.

Radionuclide purity with regard to germanium-68 breakthrough was determined after the decay of the ^{68}Ga -labelled product (minimum decay time of 48 h) using γ -ray spectrometry (Mucha, Raytest, Straubenhardt, Germany). The determination of the germanium-68 content was based on the measurement of the counts/sec with 511 ± 50 keV and a calibrated source of germanium-68.

The pH of the final product was determined by use of pH indicator strips (1.09526.0003, Universal indicator, Merck). After the decay of the ^{68}Ga -labelled product, additional pH measurements were performed using an Orion Dual Star pH-meter (Thermo Fischer, Massachusetts, United States of America), to assess the pH with and without addition of 1 M sodium bicarbonate.

Bacterial endotoxins were tested using the Endosafe nextgen-PTS Endotoxin reader and PTS-20F Limulus Amebocyte Lysate (LAL) cartridges with sensitivity of 5-0.05 EU/mL (Charles River Laboratories, Massachusetts, USA).

The sterility of the final product was tested according to the European Pharmacopoeia.

To compare the mean values of the HPLC results obtained using different reaction conditions, an independent samples t-test with equal variances and significance level of $\alpha = 0.05$ was used. To account for multiple comparisons, a Bonferroni correction was applied. All calculations were performed using Microsoft Excel (Microsoft, Redmond, USA).

Receptor binding Assays

For receptor binding assays, A431 human epidermoid carcinoma cells (American Type Culture Collection, Manassas, VA, USA) overexpressing human CCK2R through stable transfection with plasmid PCR3.1 were used. This cell-line was originally provided by Dr. Luigi Aloj [19]. All cell culture media and reagents were purchased from Invitrogen Corporation (Lofer, Austria) or Sigma-Aldrich (Darmstadt, Germany). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) of 100x penicillin-streptomycin-glutamine mixture and maintained in a humidified atmosphere containing 5% CO_2 at 37°C . The cell cultures were passaged three times per week using 5 mL 2.5% trypsin-EDTA-solution at a 1:2 or 1:3 ratio. The receptor affinity of Lu-, Ga-, Fe-, Zn-, and non-complexed DOTA-MGS5, was competitively evaluated against [^{177}Lu]Lu-DOTA-MGS5 (30,000–40,000 cpm; <0.5 nM). Assays were performed with 200,000 to 400,000 cells per well. Twelve concentrations of each test compound, ranging from 0.001 to 1000 nM (final assay concentration) were tested. All dilutions were prepared in HEPES buffer (20 mM, pH = 7.3).

The assay was performed in 96 filter well plates. Plates were first primed with ice cold Tris(hydroxymethyl)aminomethane (Tris) buffer (10 mM TRIS, 139 mM NaCl, pH = 7.3) and the buffer was removed by vacuum filtration before addition of the assay components. The test compounds were then added in triplicates (50 μL per well), followed by the radioligand (50 μL per well), and in the last step the cells (100 μL per well) were added in 20 mM HEPES buffer containing 0.5% bovine serum albumin, 10 mM MgCl_2 and 14 nM bacitracin. The plates were incubated for 60 minutes at room temperature. The reaction was terminated by vacuum filtration followed by rapid rinsing of all wells twice with 150 μL ice cold Tris buffer. The filters were collected and transferred into γ -counter tubes, and the radioactivity was measured using a γ -counter (Hidex, Turku, Finland). In addition, three tubes with 50 μL [^{177}Lu]Lu-DOTA-MGS5 solution were measured as a standard.

Half-maximal inhibitory concentration (IC₅₀) values were calculated using nonlinear regression (4 parameter logistic curve, Graphpad Prism Software, Version 11.0.0).

Results

Automated synthesis and quality control

The acceptance criteria and analytical procedures for the quality control of [⁶⁸Ga]Ga-DOTA-MGS5 and [¹⁷⁷Lu]Lu-DOTA-MGS5 specified in Table 1 and Table 2 were based on the specifications defined in Ph. Eur. monographs for other ⁶⁸Ga-labelled and ¹⁷⁷Lu-labelled radiopharmaceuticals (“Gallium (⁶⁸Ga) edotreotide injection (2482)”, “Gallium (⁶⁸Ga) oxodotreotide injection (3050)”, “Gallium (⁶⁸Ga) PSMA-11 injection (3044)”, “Lutetium (¹⁷⁷Lu) zavadotide guraxetan injection (3170)”).

Nine [⁶⁸Ga]Ga-DOTA-MGS5 batches were produced using a ⁶⁸Ge/⁶⁸Ga-generator with a germanium-68 activity of 1850 MBq at the calibration date and an elution yield of > 60% within the shelf-life of 12 months. The synthesis process took about 24 min and yielded in a mean total activity of 710 ± 117 MBq for the final product at the end of synthesis. Without addition of a radical scavenger, the percentage of the principal peak in the radiochromatogram was 90.1 ± 0.8% ($t_R = 10.0$) revealing the formation of 9.9 ± 0.8% radiochemical impurities due to radiolytic side products with relative retention time (RRT) of 0.7–1.2 of the principal peak. Instant TLC analysis performed with two different solvent systems, confirmed low levels of free gallium-68 and radiocolloid, with a percentage of radioactive species of 1.4 ± 0.3% with $R_f > 0.8$ in system 1 and of 1.0 ± 0.4% with $R_f < 0.3$ in system 2 (Fig. 2A). The addition of the radical scavengers ethanol and ascorbic acid decreased the formation of side products. The addition of 3% ethanol in the reaction solution significantly increased the percentage of the principle peak to 93.7 ± 0.6% reducing the percentage of radiolytic side products to 6.3 ± 0.6% (ethanol vs. no scavenger; $p < 0.004$). A more pronounced effect was obtained with the addition of 0.1% ascorbic acid, reaching a percentage of the principle peak of 96.9 ± 0.4% reducing the percentage of radiolytic side products to 3.1 ± 0.4% (ascorbic acid vs. no scavenger; $p < 0.001$; see Fig. 3A). TLC analysis showed comparable results with and without addition of radical scavengers. The detailed results of all quality control parameters are shown in Table 1.

Table 1
Specifications and results for the quality control of [⁶⁸Ga]Ga-DOTA-MGS5

Parameter	Method	Acceptance criteria	Results no Scavenger (n = 3)	Results Ethanol 3% (n = 3)	Results Ascorbic acid 0.1% (n = 3)
Appearance	Visual inspection	Clear, colourless solution, free of visible particles	Conforms	Conforms	Conforms
Activity of the final product	Gamma-counter, post production	> 100 MBq	674 ± 129 MBq	726 ± 132 MBq	758 ± 105 MBq
Radioactivity Concentration	Activity / volume	< 185 MBq/mL	72 ± 15 MBq/mL	76 ± 20 MBq/mL	73 ± 10 MBq/mL
Radionuclide identity	Gamma-ray spectrometry	511 keV; 1022 KeV	Conforms	Conforms	Conforms
Radionuclide purity	Germanium-68 breakthrough Gamma-ray spectrometry	Ge-68: ≤0.001% (2 h p.p.)	< 0.0001	< 0.0002%	< 0.0001
Volume	Net weight	≤ 15 mL	9.0 ± 0.3 mL	9.7 ± 1.1 mL	10.4 ± 1.1
pH of product	Indicator strip	4–7	4	4	4
Identity of [⁶⁸ Ga]Ga-DOTA-MGS5	HPLC t _R compared to Ga-DOTA-MGS5	t _R : 10 ± 1 min RRT: 0.9–1.1	t _R : 10.0 min RRT: 1.02	t _R : 10.0 min RRT: 1.02	t _R : 10.0 min RRT: 1.02
DOTA-MGS5 and metal complexes thereof	HPLC (UV) calibration curve	≤ 50 µg/Vial	23 ± 4 µg/Vial	37 ± 8 µg/Vial	34 ± 6 µg/Vial
Apparent specific activity	Radioactivity/ peptide mass	> 10 MBq/µg	32 ± 8 MBq/µg	20 ± 5 MBq/µg	23 ± 6 MBq/µg
Peak due to [⁶⁸ Ga]Ga-DOTA-MGS5	Principle peak (radio-HPLC)	≥ 95%	90.1 ± 0.8%	93.7 ± 0.6%	96.9 ± 0.4%
Radiolytic side products	Sum of the % of the peaks with RRT 0.7–1.2 (radio-HPLC)	≤ 5%	9.9 ± 0.8%	6.3 ± 0.6%	3.1 ± 0.4
Radioactive impurity (free ⁶⁸ Ga)	TLC system 1 (0.1 M NaCit pH 5): R _f 0.7–1.0)	≤ 2%	1.4 ± 0.3%	0.7 ± 0.4%	1.1 ± 0.3
Radioactive impurity (radio-colloid)	TLC system 2 (1 M NH ₄ OAc/MeOH; 1/1): R _f 0–0.3	≤ 3%	1.0 ± 0.4%	0.6 ± 0.3%	1.1 ± 0.2
Bacterial Endotoxins	LAL test	< 175 EU/Vial	< 25 EU/Vial	< 25 EU/Vial	< 50 EU/Vial

Parameter	Method	Acceptance criteria	Results	Results	Results
			no Scavenger (n = 3)	Ethanol 3% (n = 3)	Ascorbic acid 0.1% (n = 3)
Sterility	Ph. Eur.	No growth	conforms	conforms	conforms

Only with the addition of ascorbic, resulting in a concentration of 0.1% in the final formulation, a percentage of the principle peak of 95% was achieved. By means of repeated HPLC and TLC analysis a shelf-life of 2 h was defined for [^{68}Ga]Ga-DOTA-MGS5 prepared using these conditions. The stability of the formulation without additives showing a percentage of the principle peak below 95% after preparation was not tested and also with 3% ethanol used as scavenger a percentage of the principle peak > 95% could not be reached. Representative radiochromatograms of [^{68}Ga]Ga-DOTA-MGS5 prepared using different conditions are shown in Fig. 4.

Three [^{177}Lu]Lu-DOTA-MGS5 batches were produced using a mean starting activity of 8592 ± 1258 MBq. The synthesis process took about 30 min and yielded a mean total activity of 7371 ± 1190 MBq for the final product at the end of synthesis. The used reaction conditions resulted in an ascorbic acid concentration of 3% in the reaction solution. Under these conditions, the percentage of the principle peak in the radiochromatogram was $97.5 \pm 0.3\%$ together with minor formation of radiochemical impurities due to radiolytic side products of $2.5 \pm 0.3\%$ with relative retention time (RRT) of 0.7–1.2 of the principal peak. TLC analysis performed with the two different solvent systems, confirmed low levels of free lutetium-177 and radiocolloid, with a percentage of radioactive species of $0.6 \pm 0.6\%$ with $R_f > 0.8$ in system 1 and of $0.1 \pm 0.03\%$ with $R_f < 0.2$ in system 2. Representative TLC runs are shown in Fig. 2B. [^{177}Lu]Lu-DOTA-MGS5 formulated in physiological saline containing 0.01% calcium trisodium pentetate and 1.5% ascorbic acid resulted to be very stable during storage. No significant rise in radiolytic side products was observed during storage at room temperature for up to 24 h, resulting in a percentage of the principle peak of $97.3 \pm 0.6\%$ after 4 h and $97.1 \pm 0.6\%$ after 24 h (Fig. 5). Radiolytic side products remained of low levels of $2.7 \pm 0.6\%$ after 4h and $2.9 \pm 0.6\%$ after 24 h (Fig. 3B). No considerable increase in free lutetium-177 was found also in the iTLC analysis performed up to 24h after preparation. The detailed results of all quality control parameters are shown in Table 2.

Table 2
Specifications and results for the quality control of [¹⁷⁷Lu]Lu-DOTA-MGS5

Parameter	Method	Specifications	Results
Appearance	Visual inspection	Clear, colourless solution, free of visible particles	conforms
Activity of the final product	MBq	> 1000 MBq	7371 ± 1190 MBq/mL
Radioactivity Concentration	Activity / volume	< 555 MBq/mL	570 ± 111 MBq/mL
Radionuclide identity	Gamma-ray spectrometry	113 and 208 kev	conforms
Volume	Graduated vial	≤ 15 mL	13.0 ± 1.2 mL
pH of product	Indicator strip	4–7	6
Identity of [¹⁷⁷ Lu]Lu-DOTA-MGS5	HPLC t _R compared to Lu-DOTA-MGS5	t _R : 10.0 ± 1 min RRT: 0.9–1.1	t _R : 9.5 min RRT: 1.02
DOTA-MGS5 and metal complexes thereof	HPLC (UV) calibration curve	≤ 100 µg/Vial	87 ± 7 µg/Vial
Apparent specific activity	Activity/mass of peptide	> 20 MBq/µg	86 ± 19 MBq/µg
Peak due to [¹⁷⁷ Lu]Lu-DOTA-MGS5	Principle peak (radio-HPLC)	≥ 95%	97.0 ± 0.3%
Radiolytic side products	Sum of the % of the peaks with RRT 0.7–1.2 (radio-HPLC)	≤ 5%	2.5 ± 0.3%
Radioactive impurity (free ¹⁷⁷ Lu)	TLC system 1 (0.1 M NaCit pH 5): R _f 0.7-1.0)	≤ 2%	0.6 ± 0.6%
Radioactive impurity (radio-colloid)	TLC system 2 (1 M AmAc/MeOH; 1/1): R _f 0-0.3	≤ 3%	0.1 ± 0.03%
Bacterial Endotoxins	LAL test	< 175 EU/Vial	< 40 EU/Vial
Sterility	Ph. Eur.	sterile	conforms

In process controls for all synthesized batches included a cassette test confirming the integrity of the tubes and valves tested, track of the temperature and activity in the reactor during the synthesis, as well as a pressure hold test confirming the integrity of the sterile filter at the end of the process.

The mean pH of the final product as measured using pH indicator strips was 4 for [⁶⁸Ga]Ga-DOTA-MGS5 formulations and 6 for [¹⁷⁷Lu]Lu-DOTA-MGS5 formulations. For [⁶⁸Ga]Ga-DOTA-MGS5 the pH of the product was measured also using a pH meter obtaining a mean value of pH 4.0 ± 0.2 for the nine batches produced. The pH could be adjusted to pH 6.5 ± 0.2 by the addition of 1 mL of 1 M sodium bicarbonate. Radionuclide identity was confirmed by γ-ray spectrometry. The germanium-68 breakthrough of [⁶⁸Ga]Ga-DOTA-MGS5 formulations was less than 0.0002% as calculated for the end of the shelf-life of 2 h after preparation.

Endotoxin testing confirmed low levels of bacterial endotoxin of < 50 EU/vial. Sterility testing after a decay time corresponding to 10 half-lives of the radionuclide confirmed the sterility of the final product.

Identification of Ga-DOTA-MGS5, Lu-DOTA-MGS5 and other metal-complexes

The different metal-complexes were prepared with a purity of > 95%. The relative retention times (RRT) compared to DOTA-MGS5 and the resolution (R_s) compared to the preceding peak were determined by HPLC method 1 and 2 for DOTA-MGS5 and all metal complexes. The results of the identification by mass spectrometry and the mean RRT obtained for three individual runs in the two HPLC methods is summarized in Table 3.

Table 3 Identification of DOTA-MGS5 and the different metal-complexes by mass spectrometry and HPLC (method 1)											
Peptide conjugate	Calculated mass		Found mass	HPLC method 1				HPLC method 2			
	m/z [M + H] ⁺	m/z [M + 2H] ²⁺	m/z [M + 2H] ²⁺	Position	t _R	RRT	R _s	Position	t _R	RRT	R _s
Lu-DOTA-MGS5 (n = 5)	1621.6	811.3	811.5	1	9.34	0.97	N/A	4	24.15	1.13	0.64
Fe-DOTA-MGS5 (n = 3)	1502.6	751.8	752.6	2	9.41	0.97	0.35	5	24.62	1.16	0.69
DOTA-MGS5 (n = 5)	1449.7	725.3	725.7	3	9.67	1.00	1.24	1	21.29	1.00	N/A
Ni-DOTA-MGS5 (n = 3)	1505.6	753.3	752.2	4	9.75	1.01	0.42	7	25.51	1.19	0.22
Cu-DOTA-MGS5 (n = 3)	1511.0	756.0	756.0	5	9.76	1.01	0.03	6	25.39	1.19	1.05
Ga-DOTA-MGS5 (n = 5)	1516.6	758.8	758.5	6	9.84	1.02	0.37	2	22.81	1.08	2.61
Zn-DOTA-MGS5 (n = 3)	1511.6	756.3	756.3	7	9.90	1.02	0.34	3	23.80	1.12	1.48

t_R: retention time; RRT: retention time relative to DOTA-MGS5; R_s: resolution to precedent peak

In HPLC method 1 with the Avantor ACE column, DOTA-MGS5 and the different metal-complexes eluted with t_R between 9.3 and 9.9 min with the following order: Lu-DOTA-MGS5, Fe-DOTA-MGS5, DOTA-MGS5, Ni-DOTA-MGS5, Cu-DOTA-MGS5, Ga-DOTA-MGS5, and Zn-DOTA-MGS5 (Fig. 6). The resolution between adjacent peaks as presented in detail in Table 3 did not allow for baseline separation of all the peaks, did however allow to separate

Lu-DOTA-MGS5 from DOTA-MGS5 with a resolution of 1.7, whereas no baseline separation was possible for Ga-DOTA-MGS5 and DOTA-MGS5 achieving only a resolution of 0.9. Furthermore, no clear distinction of Lu/Fe-DOTA-MGS5 and Ni/Cu/Ga/Zn-DOTA-MGS5 was possible.

HPLC method 2 with the Nucleodur HTec column allowed for a better separation between peaks which eluted with t_R between 21.3 and 25.5 min, however with a different elution order: DOTA-MGS5, Ga-DOTA-MGS5, Zn-DOTA-MGS5, Lu-DOTA-MGS5, Fe-DOTA-MGS5, Cu-DOTA-MGS5, and Ni-DOTA-MGS5 (Fig. 7). A resolution > 2 could be achieved for Lu-DOTA-MGS5, DOTA-MGS5 and Ga-DOTA-MGS5. Due to the different elution order the presence of Cu/Ni-DOTA-MGS5 in the final formulation could be excluded and a clear distinction of Ga-DOTA-MGS5 and Zn-DOTA-MGS5 could be obtained. In this system the distinction of Zn/Lu/Fe-DOTA-MGS5 was difficult, as no clear baseline separation of these peaks was possible.

However, combining the results obtained with both HPLC methods, it was possible to identify the different metal complexes in the final formulation.

When analyzing the final formulation obtained from the automated [^{68}Ga]Ga-DOTA-MGS5 synthesis using HPLC method 1, three peaks were detectable. The first peak was assigned to Fe-DOTA-MGS5 (RRT vs. DOTA-MGS5: 0.97), the second to DOTA-MGS5 (RRT: 1), and the third was possibly matched to Ni-, Cu-, Ga-, or Zn-DOTA-MGS5 (RRT: 1.02). The additional information from HPLC method 2 allowed to confirm the presence of four peptide related peaks: DOTA-MGS5 (RRT: 1.00), Zn-DOTA-MGS5 (RRT: 1.12), Fe-DOTA-MGS5 (RRT: 1.16), as well as Ga-DOTA-MGS5 (RRT: 1.09), which was detectable in two analyzed samples. By performing an automated synthesis without [^{68}Ga]GaCl₃, the process related formation of Fe-DOTA-MGS5 and Zn-DOTA-MGS5 could be confirmed (Supplementary Table 1).

When analyzing the final formulation for [^{177}Lu]Lu-DOTA-MGS5 three peaks were found with HPLC method 1. The first peak was assignable to Fe- or Lu-DOTA-MGS5 (RRT vs. DOTA-MGS5: 0.97), the second to DOTA-MGS5 (RRT: 1.00), whereas the third was possibly matched to Ni-, Cu-, Ga-, or Zn-DOTA-MGS5 (RRT: 1.02). The additional information from HPLC method 2 allowed to confirm the presence of DOTA-MGS5 (RRT: 1:00) and identify Zn-DOTA-MGS5 (RRT: 1.12). However, the last peak (RRT: 1.15) was again assigned to Lu- or Fe-DOTA-MGS5. By performing an automated synthesis without [^{177}Lu]LuCl₃, the process related formation of Fe-DOTA-MGS5 could be confirmed (Supplementary Table 2).

Characterization of radiolytic side products

To identify the radiolytic side products present in the [^{68}Ga]Ga-DOTA-MGS5 formulation, Ga-DOTA-MGS5 was subjected to x-ray irradiation to mimic radiolysis. A representative HPLC analysis with HPLC method 1 showing the radiochromatogram of [^{68}Ga]Ga-DOTA-MGS5 and the UV trace of Ga-DOTA-MGS5 before and after irradiation is shown in Fig. 8, confirming the formation of similar oxidative side products. The same RRTs vs. the principle peak were found in the radiochromatogram and in the UV trace of the sample subjected to x-ray irradiation. Several peaks were observed: peaks 1–4 before the main product, the main product (peak 5) and peak 6. The peaks were manually collected and subjected to high-resolution mass spectrometry for structural characterization.

The LC-MS/MS analysis confirmed that radiolysis primarily induced oxidation of tyrosine (Tyr) at position 3 and tryptophan (Trp) at position 5 and of the linear peptide sequence. Peak 1 (RRT = 0.84) and peak 2 (RRT = 0.88) were identified as di-oxidized and mono-oxidized Ga-DOTA-MGS5, respectively (calculated m/z [$M + 2H$]²⁺: 774.28

and 766.28; observed m/z: 774.29 and 766.29). MS/MS fragmentation localized the single or double oxidation, corresponding to mass increases of 16.0 and 32.0 Da, respectively, to the tryptophan residue. Peak 3 (RRT = 0.95) and peak 4 (RRT = 0.98) were identified as different isomers of the mono-oxidized and di-oxidized peptide, respectively (calculated m/z [M + 2H]²⁺: 766.28 and 774.28; observed m/z: 766.29 and 774.29). While having the same mass as peak 2, its different retention time and MS/MS fragmentation pattern confirmed that the mass increase of 16.0 occurred on the tyrosine residue. In the di-oxidized species (peak 4), the two + 16.0 Da mass shifts were localized to the tyrosine and tryptophan residues. Peak 5, corresponding to the main peak in the chromatogram (RRT = 1.00), was confirmed to be intact Ga-DOTA-MGS5 (calculated m/z [M + 2H]²⁺: 758.28, observed m/z [M + 2H]²⁺: 758.29). Peak 6, after the main peak with RRT = 1.06 (found m/z [M + H]¹⁺: 700.35), could not be assigned to a radiolytic side product of Ga-DOTA-MGS5, despite detailed analysis of its MS/MS fragmentation pattern. As only very low levels < 1% of this peak were present in the final formulation no further characterization of this peak was carried out.

A comprehensive summary of the mass spectrometry data for all identified species is provided in Supplemental Figure S1.

Receptor affinity of DOTA-MGS5 and different metal complexes

Competitive binding assays were performed for DOTA-MGS5 and the metal-complexes identified in the final formulations of [⁶⁸Ga]Ga-DOTA-MGS5 and [¹⁷⁷Lu]Lu-DOTA-MGS5. For all tested compounds, DOTA-MGS5, Ga-DOTA-MGS5, Lu-DOTA-MGS5, Fe-DOTA-MGS5, and Zn-DOTA-MGS5 a high CCK2R affinity in the low nanomolar range could be confirmed. The detailed IC₅₀ values are summarized in Table 4 and representative binding curves are presented in Fig. 9.

Table 4
IC₅₀ values determined on A431-CCK2R cells for DOTA-MGS5 and different metal complexes against [¹⁷⁷Lu]Lu-DOTA-MGS5

Peptide	IC ₅₀
Lu-DOTA-MGS5	1.28 ± 0.6
Ga-DOTA-MGS5	1.77 ± 0.2
Fe-DOTA-MGS5	1.53 ± 0.4
Zn-DOTA-MGS5	1.24 ± 0.2
DOTA-MGS5	0.82 ± 0.2

Discussion

In this study, an optimized cassette-based synthesis process of [⁶⁸Ga]Ga-DOTA-MGS5 and [¹⁷⁷Lu]Lu-DOTA-MGS5 was established using a customized Modular-Lab PharmTracer setup, the peptide precursor DOTA-MGS5 in GMP-quality, licensed radionuclide sources and in-house produced reaction buffers. The simplified production process eliminates time consuming purifications steps and requires only sterile filtration during final formulation.

Comprehensive quality control analyses, including radio-HPLC, radio-TLC, pH, endotoxin, sterility, and gamma spectrometry testing, were performed to assess the quality of both radiopharmaceuticals. Validation runs using a set of three master batches for each reaction condition tested enabled the identification of optimal reaction parameters allowing to meet the predefined acceptance criteria. In our previous publications, we reported on automated, cassette-based production methods for [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5, incorporating a post labelling purification step using a Sep-Pak® Light C18 cartridge [1, 2] with total production time of 35 and 45 minutes, respectively. Both processes resulted in radiopharmaceuticals with high radiochemical purity and stability, meeting the quality control acceptance criteria which were deduced from pharmacopoeia monographs available for similar radiopharmaceuticals [20, 21].

One objective of this work was to establish a streamlined workflow that eliminates the need for post-labeling purification. Within the present work, we have successfully adapted the recently described synthesis approach for [^{177}Lu]Lu-PSMA-I&T and [^{177}Lu]Lu-DOTA-TATE at our department omitting SPE purification, to the production of [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 [17]. A critical consideration in developing the method was ensuring high product quality and guarantee radiochemical stability. An increased formation of radiolytic side products was observed when ^{68}Ga -labelling was performed using a sodium acetate buffer without the addition of a radical scavengers. Nearly 10% of peptide-related side products were observed in the radio-HPLC analysis performed immediately after preparation. To mitigate this, we evaluated the efficacy of two different scavengers. The addition of ethanol has previously been shown to have a pronounced radioprotective effect. Meisenheimer et al. demonstrated that within [^{68}Ga]Ga-DOTA-TOC automated synthesis, addition of 5% (v/v) ethanol reduced radiolytic degradation from $5.87 \pm 1.08\%$ to $1.03 \pm 0.47\%$ ($p < 0.0001$) [22]. Thus in our previous reported automated synthesis process for [^{68}Ga]Ga-DOTA-MGS5 based on the concentration and purification of the ^{68}Ga -eluate using a SCX cartridge and a 4.9 M NaCl/0.14 M HCl solution for elution of gallium-68 from the cartridge into the reactor, 200 μl of ethanol were added to the reaction buffer. The new method described in this work involves the direct elution of the generator into the reactor, a process analogous to using a cold kit in the preparation of ^{68}Ga -labelled radiopharmaceuticals [23]. Several cold kits with marketing authorisation are available for the production of [^{68}Ga]Ga-DOTA-TOC, [^{68}Ga]Ga-DOTA-TATE and [^{68}Ga]Ga-PSMA-11 [24]. The addition of 0.1% (v/v) ascorbic acid in the process reliably ensured the principal peak due to [^{68}Ga]Ga-DOTA-MGS5 in the HPLC radiochromatogram remained above our internal release criterion of 95% and guaranteed a shelf-life of the product of 2 h post preparation. The addition of 3% (v/v) ethanol was significantly less effective and only conferred a minor improvement, however with the purity of the final product dropping below the acceptable threshold after two hours. The process also included a possible pH adjustment step increasing the pH of the final formulation to 6–7 and allowing to match physiological conditions. For [^{177}Lu]Lu-DOTA-MGS5, which was prepared using a reaction buffer based on ascorbic acid [1, 25] and formulated in physiological saline additionally containing 0.9 mg DTPA and 150 mg ascorbic acid with a pH of 6, a high product quality and exceptional stability was achieved, with the principal peak due to [^{177}Lu]Lu-DOTA-MGS5 remaining well above 95% even after 24 hours of storage. Omitting the SPE purification step resulted in a slight increase in unbound radiometal. The presence of free radiometal and radiocolloid as determined by iTLC increased from 0.9 to 2.4% for [^{68}Ga]Ga-DOTA-MGS5 and from 0.31% to 0.72% for [^{177}Lu]Lu-DOTA-MGS5 [1, 8]. This results align with previous findings by Kraihammer et al. using the same system for other ^{177}Lu -labelled radiopharmaceuticals [17]. Despite this increase, the final products for both radiopharmaceuticals were still well within the accepted safety limits.

Avoiding purification steps also enables the use of smaller single-use cassettes, thereby reducing the overall volume of radioactive waste generated during routine production. The simpler process workflow also shortens the synthesis time by eliminating cartridge activation, product fixation, and elution steps, while reducing the number of fluidic operations and potential points of failure and product loss, allowing to improve the reliability of routine automated production [17]. The new process resulted only in a minor reduction of the production time to 24–30 min, but supports an efficient and reliable routine production. Omitting the final purification step requires however a profound understanding of the necessary reaction conditions and control of all potential impurities.

Thus another objective of the present study was the comprehensive evaluation of all potential impurities. Next to the identification of the peptide precursor and the gallium/lutetium complexed peptide analogues the characterization of other peptide related peaks in the UV chromatogram was performed using different reference metal complexes. The combination of HPLC and MS analyses identified the additional presence of Fe-DOTA-MGS5, Zn-DOTA-MGS5 in the final formulation. These metal complexes were detected in both the [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 formulations. Since these metal complexes appeared in both formulations, their origin is most likely the presence of metal impurities in the synthesis cassette or reaction buffers, and not within the radionuclide sources. This was also confirmed by performing test syntheses without radionuclide sources. In [^{68}Ga]Ga-DOTA-MGS5 formulations, the detection of Zn-DOTA-MGS5 also aligns with previous observations in [^{68}Ga]Ga-DOTA-TATE formulations, in which Zn-DOTA-TATE was identified. Radzik et al. attributed the formation of this species to the generation of stable zinc-68 following the decay of gallium-68, competing for DOTA complexation [26]. This process may contribute to the formation of Zn-DOTA-MGS5 in the present study. The findings of Mu et al. are also consistent with our observations, confirming the formation of Fe- and Zn-DOTA-TATE complexes in the production of [^{68}Ga]Ga-DOTA-TATE [27]. Metals present in traces in the production process, can lead to reduced labelling yield and the presence of higher levels of free radiometal requiring a post labelling purification step [28], and also compromise the stability of the final product. For the biological characterisation of the identified metal complexes, their binding affinities were determined in a competitive assay against [^{177}Lu]Lu-DOTA-MGS5 confirming a high affinity in the low nanomolar range for all complexes. The sum of the UV peaks of the peptide precursor, the gallium/lutetium complexed peptides, as well as of the additional metal complexes identified in the formulation in addition, was used for the limit test of the peptide content based on a calibration curve.

Given the more critical impact of radiolytic impurities on product quality and subsequent clinical use for imaging and therapy, a deeper characterization of these side products was performed. For this purpose DOTA-MGS5 was complexed with the stable isotope gallium-69 and subjected to a high-dose external gamma radiation source. Comparison of the respective UV chromatogram with the radiochromatograms of the produced [^{68}Ga]Ga-DOTA-MGS5 master batches confirmed a similar pattern of oxidative side product formation. High-resolution mass spectrometry of the formed degradation products confirmed that oxidation of DOTA-MGS5 results in singly and doubly oxidized forms of the tryptophan and tyrosine residues. These amino acids are present in position 3 and position 5 of the 8 amino acid linear peptide sequence with the C-terminal sequence Trp-(NMe)Nle-Asp-1Nal-NH₂ being essential for receptor recognition. In the design of the peptide analogue oxidation prone methionine in position 6 was replaced by norleucine to avoid loss of receptor affinity due to oxidative processes [3, 29–31]. Similar to our findings, Mu et al. previously found several radioactive impurities during the production of high-dose [^{68}Ga]Ga-DOTA-TATE. LC-MS/MS analysis of these impurities revealed oxidation products characterized by the addition of one or two oxygen atoms. These were localized to the indole ring of the tryptophan residue of DOTA-

TATE [27]. Oxidation species can have a profound impact on biological activity. The oxidation of these aromatic residues disrupts essential π -stacking and hydrophobic interactions within the receptor binding pocket, altering the peptide's conformation and severely diminishing the binding affinity. Given the low levels < 3% of peptide-related radiolytic side product in the final preparations of [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 no further characterization of the receptor affinity of oxidative species was carried out.

In the final formulation of [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5 containing a low peptide mass of 50–100 μg the presence of the different identified metal complexes as well as the minor formation of peptide-related radiolytic side products is not expected to influence the image quality or therapeutic effect.

Conclusion

In this study, we successfully developed and validated a purification-free automated synthesis process for the theranostic pair [^{68}Ga]Ga-DOTA-MGS5 and [^{177}Lu]Lu-DOTA-MGS5, streamlining their production for clinical use. We systematically addressed the characterization of the potential sources of radioactive and chemical impurities: radiolytic degradation and metal contamination. Our results demonstrate that radiolytic instability, which severely compromises binding affinity, can be effectively diminished through the use of ascorbic acid as a radical scavenger. Our comprehensive impurity profiling identified additional non-radioactive DOTA-MGS5 complexes, as well as the minor formation of radiolytic side products. These minor impurities do not compromise patient safety or therapeutic efficacy in the clinical use. The established synthesis process allows the robust preparation of both radiopharmaceuticals.

Declarations

Acknowledgements

The authors would like to thank the Team of the Radiopharmacy from the Department of Nuclear Medicine, Medical University of Innsbruck for their valuable contribution maintaining equipment and taking care of routine work.

Author contributions

The author contributions are as follows: Conceptualization was led by CD, EG, and ES. The methodology was developed by EG, ES, MK, SM, and TZ. The experiments were carried out by ES, LP, SM, and TZ. KF is responsible for the MS-MS spectra measurements while PE carried out the external radiation of samples. The original manuscript was drafted by EG, ES, SM, and TZ. Visualizations were created by SM and TZ. Project administration and supervision were handled by EG and ES. All authors contributed to the writing, review, and editing, and have read and approved the final version for publication.

Funding

This study was financially supported by the Austrian Science Fund (FWF), project P34732-B. Open Access Funding was supported by the Austrian Science Fund (FWF).

Availability of data and materials

Supporting information is provided containing further data. Additional data are available from the corresponding author upon reasonable request.

Declarations Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest statement

EG are listed as an inventor on the European patent application no. 17174973. EG consults Evergreen Theragnostics, Inc. in product development. No other conflict of interest relevant to this article was reported.

References

1. Hörmann AA, Klingler M, Rangger C, Mair C, Decristoforo C, Uprimny C, et al. Radiopharmaceutical Formulation and Preclinical Testing of (68)Ga-Labeled DOTA-MGS5 for the Regulatory Approval of a First Exploratory Clinical Trial. *Pharmaceuticals (Basel)*. 2021;14. 10.3390/ph14060575.
2. Zavvar TS, Hörmann AA, Konijnenberg M, Kraihammer M, Mair C, Kronthaler A, et al. Radiopharmaceutical formulation and preliminary clinical dosimetry of [(177)Lu]Lu-DOTA-MGS5 for application in peptide receptor radionuclide therapy. *Eur J Nucl Med Mol Imaging*. 2025;52:1321–31. 10.1007/s00259-024-06979-1.
3. Reubi JC. Targeting CCK receptors in human cancers. *Curr Top Med Chem*. 2007;7:1239–42. 10.2174/156802607780960546.
4. Sanchez C, Escrieut C, Clerc P, Gigoux V, Waser B, Reubi JC, et al. Characterization of a novel five-transmembrane domain cholecystokinin-2 receptor splice variant identified in human tumors. *Mol Cell Endocrinol*. 2012;349:170–9. 10.1016/j.mce.2011.10.010.
5. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev*. 2003;24:389–427. 10.1210/er.2002-0007.
6. Uprimny C, von Guggenberg E, Sviridenko A, Bayerschmidt S, Rossetti LM, Roustaei H, et al. Diagnostic Performance of the (68)Ga-Labeled Minigastrin Analog DOTA-MGS5 in Patients with Advanced Medullary Thyroid Cancer and Other Neuroendocrine Tumors. *J Nucl Med*. 2025;66:1581–8. 10.2967/jnumed.125.269863.
7. von Guggenberg E, di Santo G, Uprimny C, Bayerschmidt S, Warwitz B, Hörmann AA, et al. Safety, Biodistribution, and Radiation Dosimetry of the (68)Ga-Labeled Minigastrin Analog DOTA-MGS5 in Patients with Advanced Medullary Thyroid Cancer and Other Neuroendocrine Tumors. *J Nucl Med*. 2025;66:257–63. 10.2967/jnumed.124.268877.

8. Zavvar TS, Santo G, Gruber L, Kronthaler A, Hagenbuchner J, Skvortsova I, et al. Paving the Way for CCK2R-Targeted Peptide Receptor Radionuclide Therapy with [(177)Lu]Lu-DOTA-MGS5 in Patients with Small Cell Lung Cancer. *Pharmaceutics*. 2026;18. 10.3390/pharmaceutics18010138.
9. Zavvar TS, Hörmann AA, Klingler M, Summer D, Rangger C, Desrues L, et al. Effects of Side Chain and Peptide Bond Modifications on the Targeting Properties of Stabilized Minigastrin Analogs. *Pharmaceutics* (Basel). 2023;16. 10.3390/ph16020278.
10. Hörmann AA, Plhak E, Klingler M, Rangger C, Pfister J, Schwach G, et al. Automated Synthesis of (68)Ga-Labeled DOTA-MGS8 and Preclinical Characterization of Cholecystokinin-2 Receptor Targeting. *Molecules*. 2022;27. 10.3390/molecules27062034.
11. Struelens L, Aalbersberg E, Beels L, Cherbuin N, D'Asseler Y, De Monte F, et al. How much do (68)Ga-, (177)Lu- and (131)I-based radiopharmaceuticals contribute to the global radiation exposure of nuclear medicine staff? *EJNMMI Phys*. 2024;11:95. 10.1186/s40658-024-00695-3.
12. Agency IAE. Occupational Radiation Protection. 11. International Atomic Energy Agency; 2018.
13. Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. *Eur J Nucl Med Mol Imaging*. 2010;37:1049–62. 10.1007/s00259-010-1407-3.
14. Boschi S, Malizia C, Lodi F. Overview and perspectives on automation strategies in (68)Ga radiopharmaceutical preparations. *Recent Results Cancer Res*. 2013;194:17–31. 10.1007/978-3-642-27994-2_2.
15. Velikyan I. 68Ga-Based radiopharmaceuticals: production and application relationship. *Molecules*. 2015;20:12913–43. 10.3390/molecules200712913.
16. Aerts J, Ballinger JR, Behe M, Decristoforo C, Elsinga PH, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice for the small-scale preparation of radiopharmaceuticals using automated modules: a European perspective. *J Label Comp Radiopharm*. 2014;57:615–20. 10.1002/jlcr.3227.
17. Kraihammer M, Decristoforo C, Knoll A, Zavvar TS, von Guggenberg E, Rangger C et al. Comparative Radiolabelling of PSMA I&T and Somatostatin Analogues with ¹⁷⁷Lu and ⁹⁰Y: The Impact of SPE Purification. 2026. <https://doi.org/10.21203/rs.3.rs-10585121/v1>
18. Krol VE, Pandey MK. In: Srivastava V, Vatsa R, editors. Targeted Radiopharmaceuticals and Imaging: Development Challenges and Opportunities. Royal Society of Chemistry; 2025. pp. 111–58.
19. Aloj L, Caracò C, Panico M, Zannetti A, Del Vecchio S, Tesauro D, et al. In vitro and in vivo evaluation of ¹¹¹In-DTPAGlu-G-CCK8 for cholecystokinin-B receptor imaging. *J Nucl Med*. 2004;45:485–94.
20. Europe Co. Gallium (68Ga) PSMA-11 injection (Monograph 3044). European Pharmacopoeia. 12th ed. Strasbourg: Council of Europe; 2025.
21. Europe Co. Lutetium (177Lu) zidovudine gaxetan (Monograph 3170). European Pharmacopoeia. 12th ed. Strasbourg: Council of Europe; 2025.
22. Meisenheimer M, Kürpig S, Essler M, Eppard E. Ethanol effects on (68)Ga-radiolabelling efficacy and radiolysis in automated synthesis utilizing NaCl post-processing. *EJNMMI Radiopharm Chem*. 2019;4:26. 10.1186/s41181-019-0076-1.
23. Mukherjee A. An Update on Extemporaneous Preparation of Radiopharmaceuticals Using Freeze-Dried Cold Kits. *Mini Rev Med Chem*. 2021;21:1322–36. 10.2174/1389557520999201214233634.

24. Lepareur N. Cold Kit Labeling: The Future of (68)Ga Radiopharmaceuticals? *Front Med (Lausanne)*. 2022;9:812050. 10.3389/fmed.2022.812050
25. Petrik M, Knetsch PA, Knopp R, Imperato G, Ocak M, von Guggenberg E, et al. Radiolabelling of peptides for PET, SPECT and therapeutic applications using a fully automated disposable cassette system. *Nucl Med Commun*. 2011;32:887–95. 10.1097/MNM.0b013e3283497188.
26. Radzik M, Pijarowska-Kruszyna J, Jaroń A, Maurin M, Decristoforo C, Mikołajczak R, et al. Development and validation of the HPLC method for quality control of radiolabelled DOTA-TATE and DOTA-TOC preparations. *Nucl Med Biol*. 2021;93:63–73. 10.1016/j.nucmedbio.2020.11.005.
27. Mu L, Hesselmann R, Oezdemir U, Bertschi L, Blanc A, Dragic M, et al. Identification, characterization and suppression of side-products formed during the synthesis of high dose ⁶⁸Ga-DOTA-TATE. *Appl Radiat Isot*. 2013;76:63–9. 10.1016/j.apradiso.2012.07.022.
28. Rizzello M, Pacelli A, De Bari MD, Cutrignelli A, Iacobazzi RM, Lopalco A, et al. (68)Ga Extemporaneous Preparations in Radiopharmacy. *Pharmaceutics*. 2025;17. 10.3390/pharmaceutics17070802.
29. Breeman WA, Fröberg AC, de Blois E, van Gameren A, Melis M, de Jong M, et al. Optimised labeling, preclinical and initial clinical aspects of CCK-2 receptor-targeting with 3 radiolabeled peptides. *Nucl Med Biol*. 2008;35:839–49. 10.1016/j.nucmedbio.2008.09.006.
30. Rangger C, Klingler M, Balogh L, Pöstényi Z, Polyak A, Pawlak D, et al. (177)Lu Labeled Cyclic Minigastrin Analogues with Therapeutic Activity in CCK2R Expressing Tumors: Preclinical Evaluation of a Kit Formulation. *Mol Pharm*. 2017;14:3045–58. 10.1021/acs.molpharmaceut.7b00241.
31. Klingler M, Summer D, Rangger C, Haubner R, Foster J, Sosabowski J, et al. DOTA-MGS5, a New Cholecystokinin-2 Receptor-Targeting Peptide Analog with an Optimized Targeting Profile for Theranostic Use. *J Nucl Med*. 2019;60:1010–6. 10.2967/jnumed.118.221283.

Figures

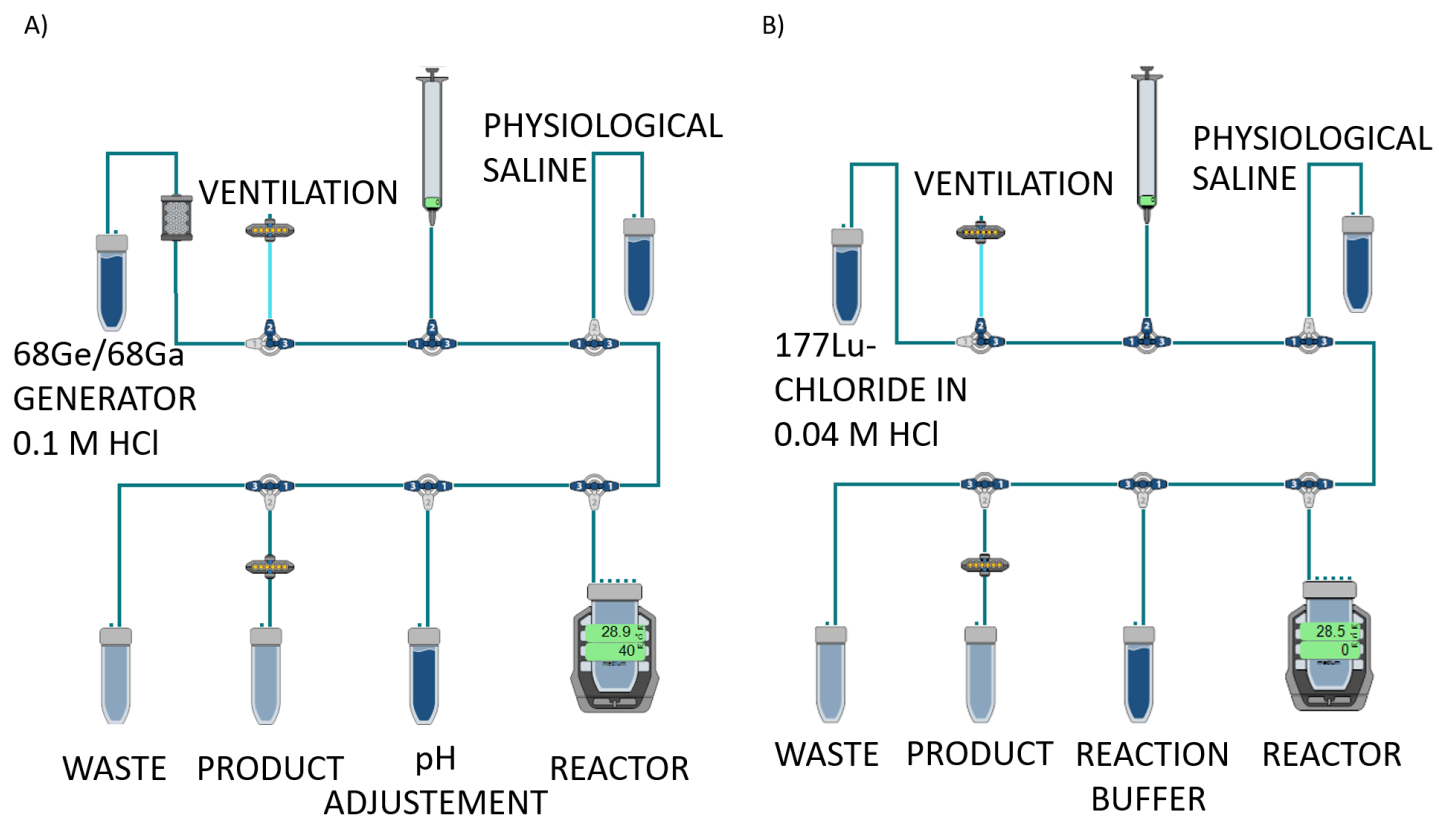


Figure 1

Synthesis scheme for the automated production of A) [^{68}Ga]Ga-DOTA-MGS5 and B) [^{177}Lu]Lu-DOTA-MGS5

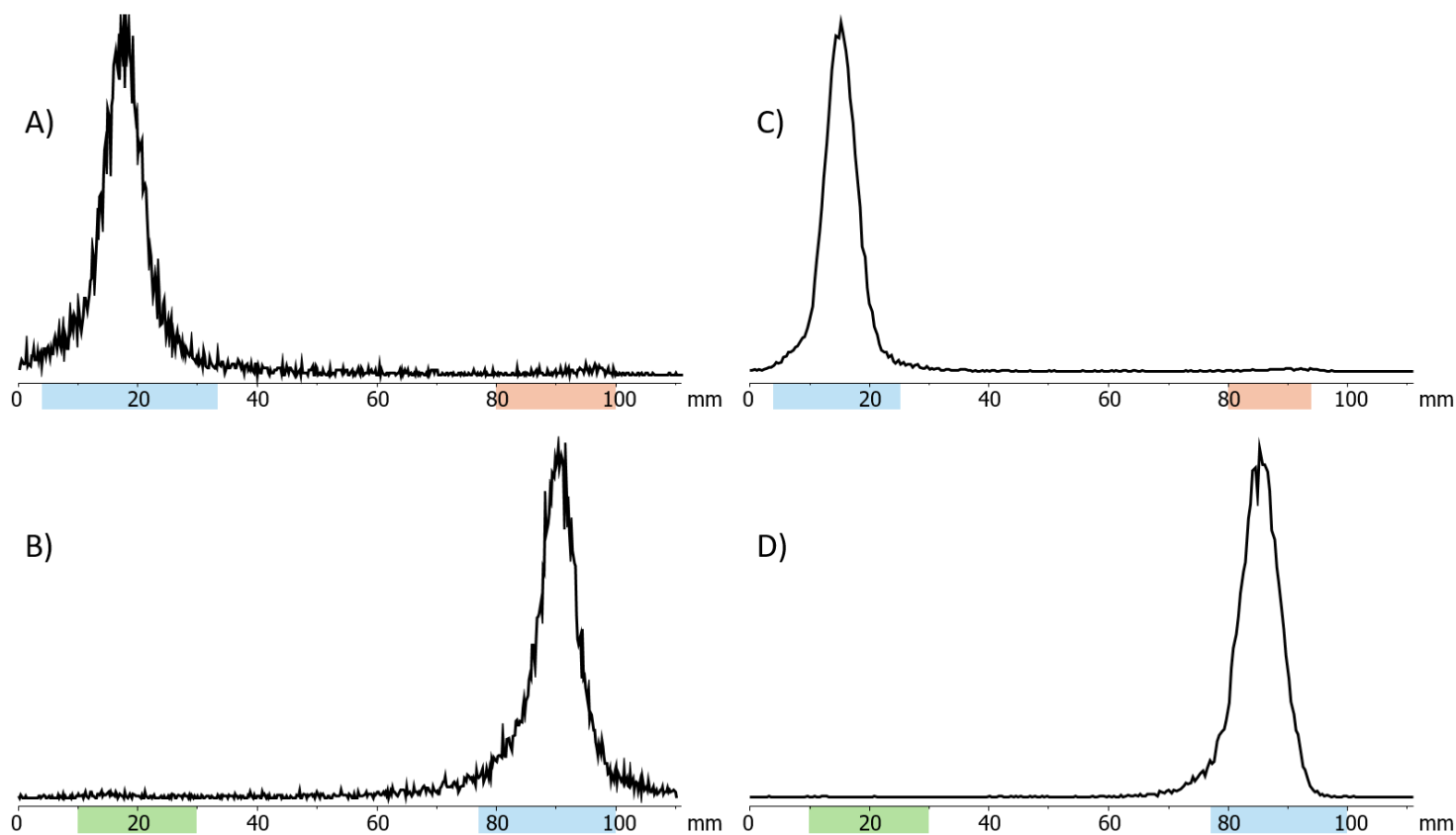


Figure 2

iTLC chromatograms of [68Ga]Ga-DOTA-MGS5 for A) system 1 and B) system 2 and of [¹⁷⁷Lu]Lu-DOTA-MGS5 for C) system 1 and D) system 2

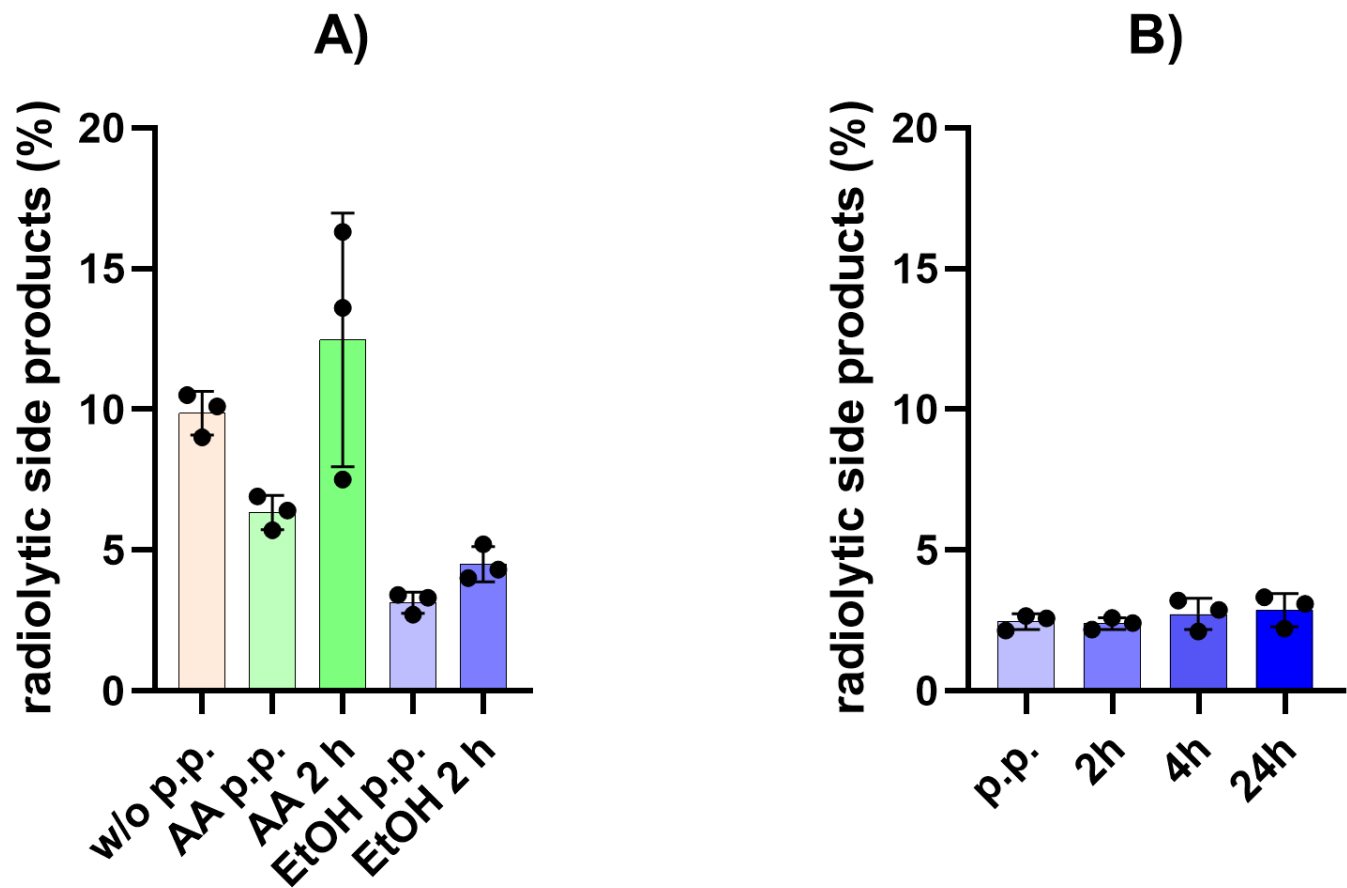


Figure 3

Percentage of radiolytic side products found for A) [68Ga]Ga-DOTA-MGS5 prepared under different reaction conditions and B) [¹⁷⁷Lu]Lu-DOTA-MGS5 as analysed post production (p.p.) and at 2-24 h after storage at room temperature; w/o=without scavenger; AA=0.1% ascorbic acid; EtOH=3% ethanol, p.p.=post production (n=3 for all)

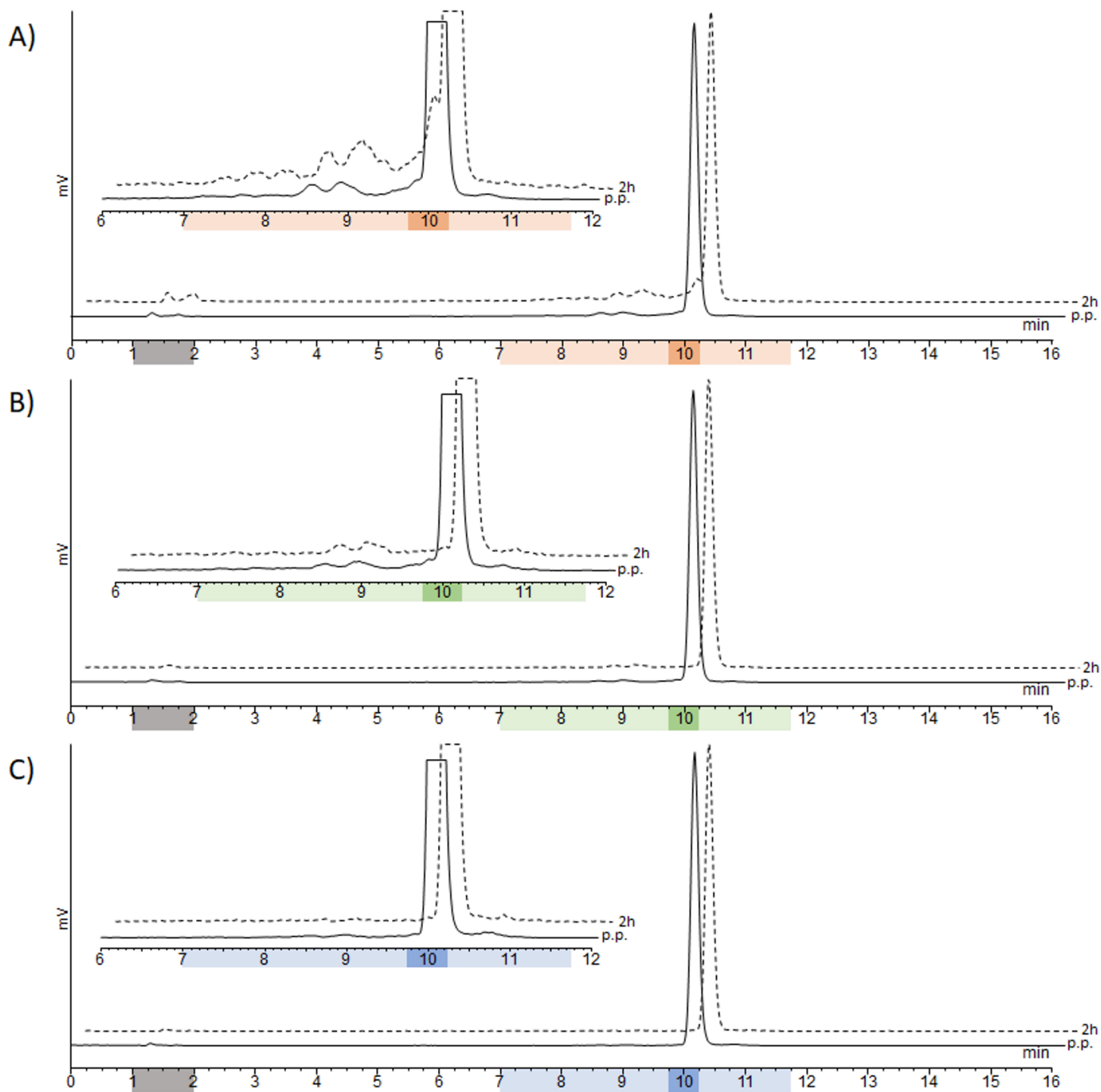


Figure 4

Radiochromatograms post preparation and after 2 h storage at room temperature of $[^{68}\text{Ga}]\text{Ga-DOTA-MGS5}$ prepared A) without scavenger, and with the addition of B) 3% ethanol and C) 0.1% ascorbic acid

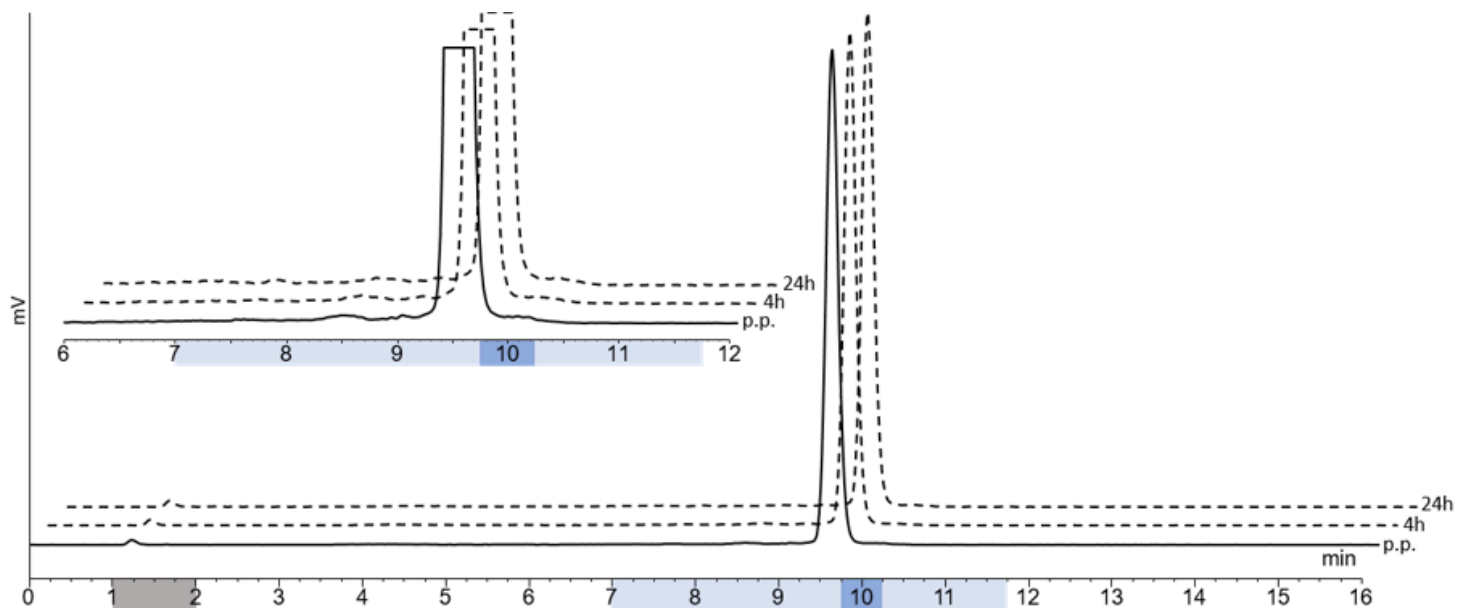


Figure 5

Radiochromatograms post preparation and after 4 and 24 h storage at room temperature of [^{177}Lu]Lu-DOTA-MGS5

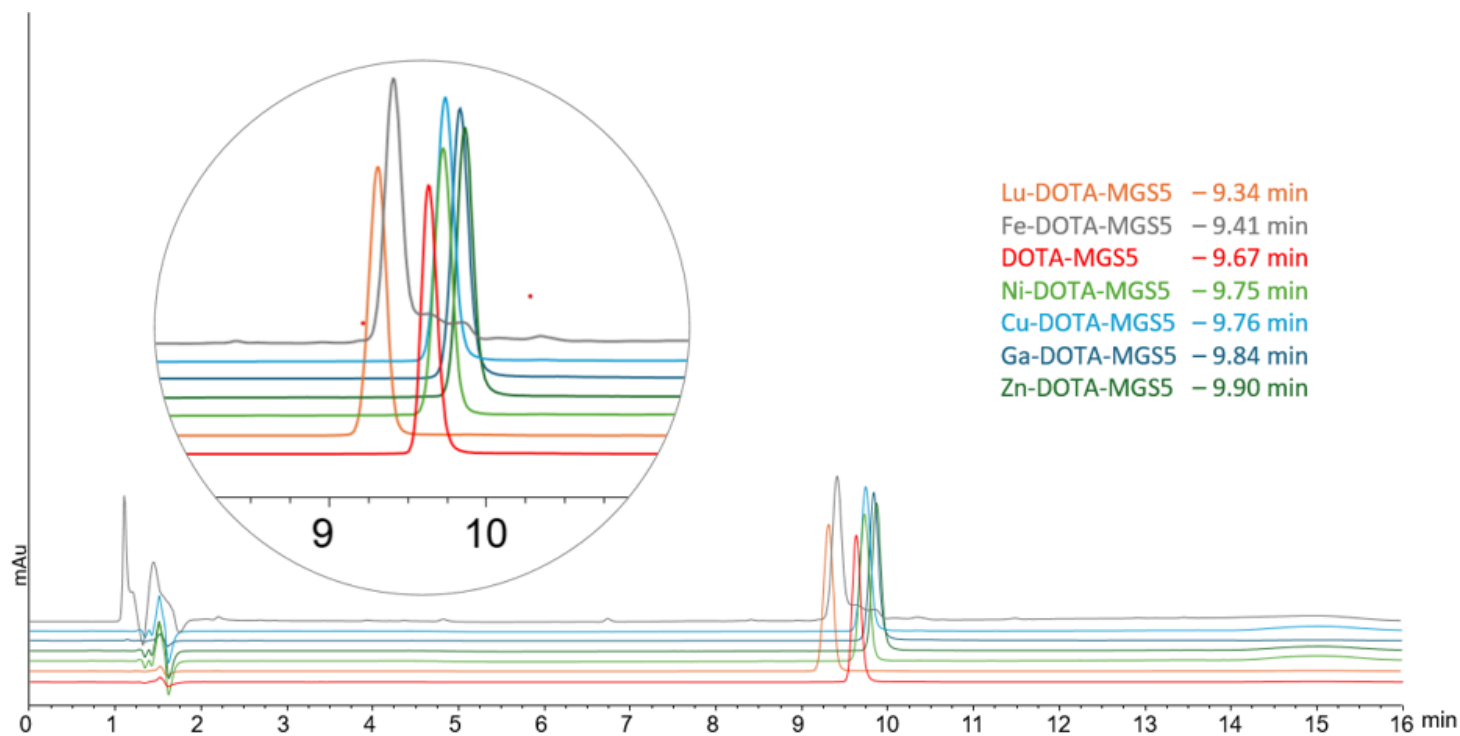


Figure 6

Representative UV-chromatograms of DOTA-MGS5 and the different metal complexes for HPLC method 1

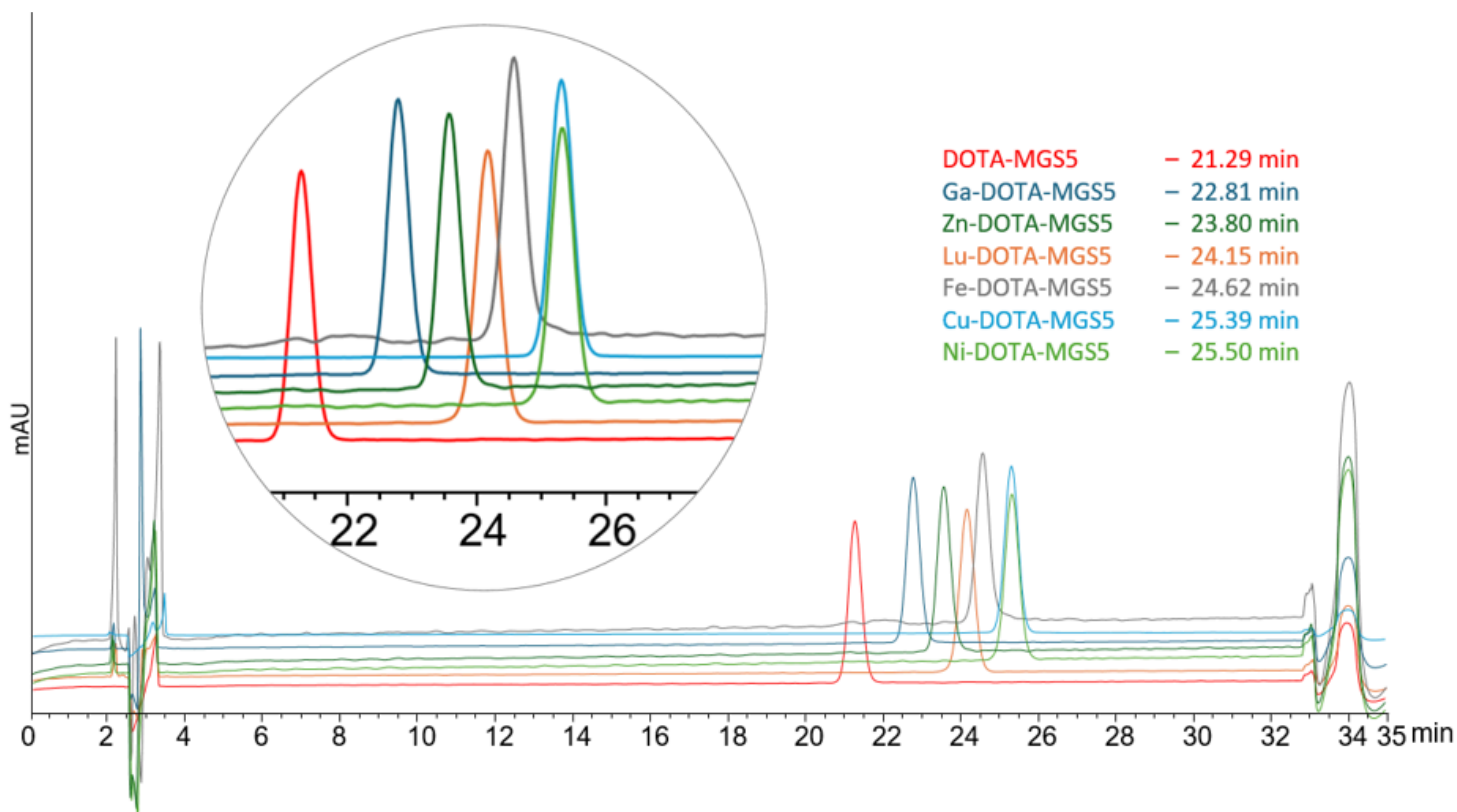


Figure 7

Representative UV-chromatograms of DOTA-MGS5 and the different metal complexes with HPLC method 2

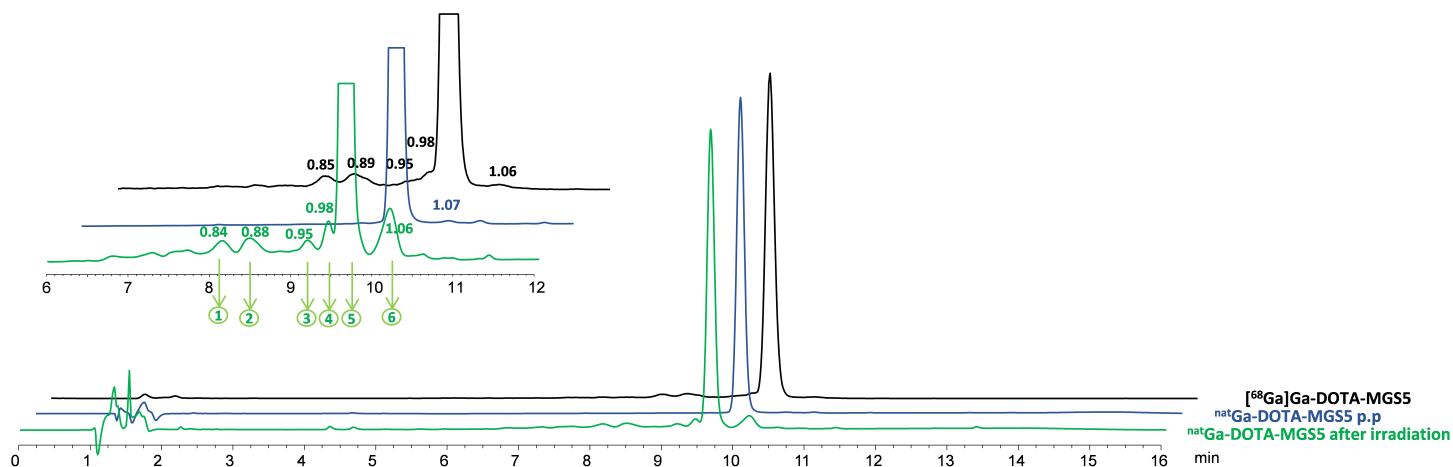


Figure 8

Representative UV-chromatograms of Ga-DOTA-MGS5 before (blue trace) and after irradiation (green trace) and radiochromatogram of [⁶⁸Ga]Ga-DOTA-MGS5 (black trace) for comparison of the formed side products. The relative retention times (RRTs) of the detected peaks vs. the principle peak are shown for each trace.

Receptor binding Assays

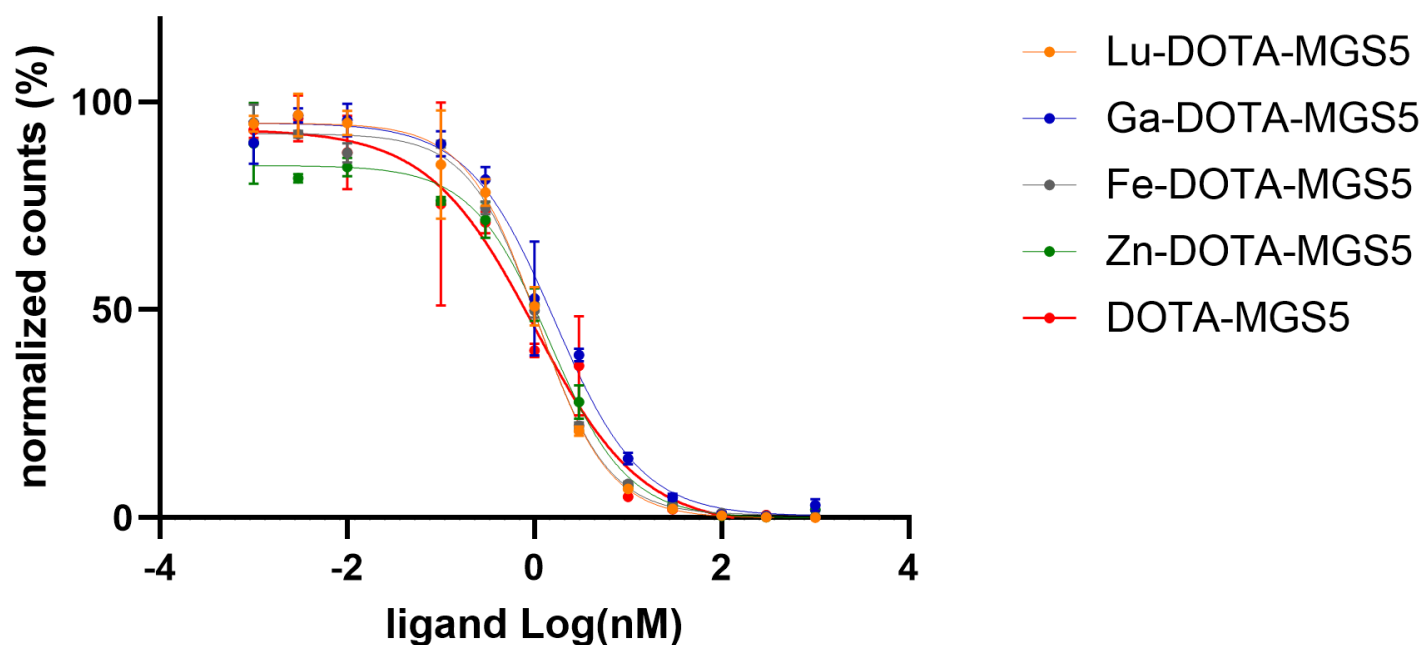


Figure 9

Representative binding curves for DOTA-MGS5 and the different metal complexes tested

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.png](#)
- [SupplementaryTable1.docx](#)
- [SupplementaryTable2.docx](#)
- [GaMGS5Kitlabelling260910supplementaryInformation.docx](#)