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Radiosynthesis of clinical doses of ^{68}Ga -DOTATATE (GalioMedix™) and validation of organic-matrix-based $^{68}\text{Ge}/^{68}\text{Ga}$ generators

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Abstract

Introduction— ^{68}Ga -DOTATATE is a radiolabeled peptide-based agonist that targets somatostatin receptors overexpressed in neuroendocrine tumors. Here, we present our results on validation of organic matrix $^{68}\text{Ge}/^{68}\text{Ga}$ generators (ITG GmbH) applied for radiosynthesis of the clinical doses of ^{68}Ga -DOTATATE (GalioMedix™).

Methods—The clinical grade of DOTATATE (25 $\mu\text{g} \pm 5\mu\text{g}$) compounded in 1MNaOAc at pH=5.5 was labeled manually with $514 \pm 218\text{MBq}$ ($13.89 \pm 5.9\text{ mCi}$) of ^{68}Ga eluate in 0.05 N HCl at 95 °C for 10 min. The radiochemical purity of the final dose was validated using radio-TLC. The quality control of clinical doses included tests of their osmolarity, endotoxin level, radionuclide identity, filter integrity, pH, sterility and ^{68}Ge breakthrough.

Results—The final dose of $272 \pm 126\text{MBq}$ ($7.35 \pm 3.4\text{ mCi}$) of ^{68}Ga -DOTATATE was produced with a radiochemical yield (RCY) of $99\% \pm 1\%$. The total time required for completion of radiolabeling and quality control averaged approximately 35 min. This resulted in delivery of $50\% \pm 7\%$ of ^{68}Ga -DOTATATE at the time of calibration (not decay corrected).

Conclusions— ^{68}Ga eluted from the generator was directly applied for labeling of DOTA-peptide with no additional pre-concentration or pre-purification of isotope. The low acidity of ^{68}Ga eluate allows for facile synthesis of clinical doses with radiochemical and radionuclide purity higher than 98% and average activity of $272 \pm 126\text{ MBq}$ ($7.3 \pm 3\text{ mCi}$). There is no need for post-labeling C18 Sep-Pak purification of final doses of radiotracer. Advances in knowledge and implications for patient care.

The clinical interest in validation of ^{68}Ga -labeled agents has increased in the past years due to availability of generators from different vendors (Eckert-Ziegler, ITG, iThemba), favorable approach of U.S. FDA agency to initiate clinical trials, and collaboration of U.S. centers with

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leading EU clinical sites. The list of ^{68}Ga -labeled tracers evaluated in clinical studies should grow because of the sensitivity of PET technique, the simplicity of the shakebake approach for the dose preparation and reliability of $^{68}\text{Ge}/^{68}\text{Ga}$ generators. Our studies have confirmed the reproducible elution profile, and high reliability of ITG GmbH generators required for routine doses preparation according to FDA recommendations.

Keywords

$^{68}\text{Ge}/^{68}\text{Ga}$ generator; ^{68}Ga -DOTATATE; ^{68}Ge breakthrough; Positron emission tomography (PET); Quality control of PET-tracers doses; Neuroendocrine tumors

1. Introduction

There is a growing interest in use of generator-produced gallium-68 (^{68}Ga) for molecular imaging of diseases [1–19]. This is primarily due to superior spatial resolution of ^{68}Ga positron emission tomography (PET) compared to single photon emission computed tomography (SPECT); advantageous nuclear properties of the ^{68}Ga isotope; and the commercial availability of clinically-useful $^{68}\text{Ge}/^{68}\text{Ga}$ generators, which allow for efficient on-demand production of the radionuclide [20]. Three types of $^{68}\text{Ge}/^{68}\text{Ga}$ generators are now available from three separate manufacturers (Eckert-Ziegler GmbH (Germany); iThemba (South Africa); and ITG GmbH (Germany)); and are currently available for clinical and research use in the European Union (EU) and the United States (US). Each of these generators shares similar theoretical-design characteristics, but differ in the composition of the immobilizing matrix for ^{68}Ga parent radionuclide, germanium-68 (^{68}Ge). Although the elution times of the various stationary phases are similar (a few minutes), differences in the stationary-phase matrix result also in differences in the strength of hydrochloric acid (HCl) that is used for ^{68}Ga elution and in the potential for co-elution (breakthrough) of ^{68}Ge and other metallic impurities. [2,16,19,20]. While the matrix of iThemba and Eckert-Ziegler generators is based on tin oxide (SnO_2) and titanium oxide (TiO_2), respectively, the adsorbent used in ITG generators consists of organic matrix immobilized on silica-resin [2,21–25]. The adsorbent inside the column consists of derivative of dodecyl gallate (Lauryl G) attached to HPLC-grade silica gel. This novel stationary phase allows for elution of ^{68}Ga using a significantly lower concentration of hydrochloric acid (0.05 M HCl) than is required by other generators [24]. In addition, the silica-based resin has lower affinity to metals such as Zn, Fe, and Ti, which putatively minimizes co-elution of metal contaminants in ^{68}Ga eluate. Herein, we describe a simplified protocol for radiosynthesis of chelator-modified somatostatin analog ^{68}Ga -DOTATATE using the organic-matrix-based $^{68}\text{Ge}/^{68}\text{Ga}$ generators (ITG GmbH). We validated the efficacy and reliability of these generators based on their performance during synthesis of more than 100 doses of ^{68}Ga -labeled tracer validated in clinical trial (IND 117289).

2. Results

The $^{68}\text{Ge}/^{68}\text{Ga}$ generators (1110 MBq, 30 mCi ITG GmbH) used in these clinical studies were manufactured under GLP conditions and have a manufacturer-guaranteed shelf life of 6 months (or 125 elutions). Generators were first used for clinical studies for 6 months — and

were also further evaluated for research applications by examination of their performance over an extended period of 8 months.

2.1. Elution profile and ^{68}Ge breakthrough

All steps from elution of the generator to labeling of clinical kits were performed manually. An understanding of the elution behavior of ^{68}Ga was developed by use of a fractional elution approach in which 1 mL volume fractions of the eluate were collected sequentially and measured for ^{68}Ga content. Using this approach it was determined that more than 90% of the total radioactivity generator-produced ^{68}Ga could be collected in the second and third 1 mL fractions, while marginal amounts of Ga activity were observed in the first and fourth fractions (Fig. 1). These main fractions were used without further purification or processing for radiolabeling of DOTATATE.

The organic-matrix based generators (loaded at the manufacturer site with 1110 MBq, 30 mCi of ^{68}Ge) produced during the first elutions 932–954 MBq (25.1–25.8 mCi of ^{68}Ga that corresponds to 84%–86% yield for a decay-corrected generator (80% without decay correction; Fig. 2). These values were consistent with the specifications of the generator provided by ITG, which guarantees over 80% of activity eluted from the generator at the time of calibration. A similar elution profile was reported for TiO_2 -based generators (Eckert-Ziegler) and SnO_2 -based generators (iThemba) [26]. The technical specifications of the ITG GmbH generators are shown in Table 1. According to the manufacturer's information, the ^{68}Ge content in ^{68}Ga eluate should not exceed 0.005% at the time of calibration. The ^{68}Ge breakthrough was at least a factor of two lower in final doses of ^{68}Ga -DOTATATE without the need for further purification via a C18 Sep-Pak column, as is often used for final purification of chelator-modified ^{68}Ga -labeled peptides. Further, our results indicate that there is no need to purify or pre-concentrate ^{68}Ga eluate to achieve acceptable radiochemical quality control objectives (e.g., radiochemical purity, specific activity, reaction efficiency).

In the course of normal laboratory operations, generators are often eluted twice daily, and as expected, the eluted ^{68}Ga radioactivity is a function of the ingrowth time allowed between elution runs. Our standard procedure permits 4 h time breaks between elutions, which allowed for regeneration over 90% of ^{68}Ga radioactivity (Table 2).

2.2. Radiolabeling of ^{68}Ga - DOTATATE clinical doses

We synthesized over 100 doses of ^{68}Ga -DOTATATE over a period of more than one year. The clinical doses of DOTATATE ($25 \mu\text{g} \pm 5 \mu\text{g}$) were compounded in 1MNaOAc at pH = 5.5 and labeled with an average of $514 \pm 218 \text{ MBq}$ ($13.89 \pm 5.9 \text{ mCi}$) of ^{68}Ga using a reaction temperature of 95°C for 10 min (Fig. 3). Reactions were conducted in the presence of ascorbic acid used as a free radical species scavenger. Our IND protocol permits release of 148–592 MBq (4–16 mCi) of ^{68}Ga -DOTATATE produced with a radiochemical yield (%RCY) of 95% (percent incorporation), as determined by iTLC. In our hands, radiosynthesis of ^{68}Ga -DOTATATE proceeded with average RCY of $99\% \pm 1\%$ and led to release of $272 \pm 126 \text{ MBq}$ ($7.35 \pm 3.4 \text{ mCi}$) of ^{68}Ga -DOTATATE in $9.1 \pm 0.3 \text{ mL}$. The presence of metallic impurities in the final dose or eluate was not determined in these

studies. This test was not required to complete the quality control of the final dose of ^{68}Ga -DOTATATE.

The total time required for completion of radiolabeling and quality control averaged approximately 35 min. This resulted in delivery of $50\% \pm 7\%$ of ^{68}Ga -DOTATATE at the time of calibration (not decay corrected) compared to the starting activity of this radiotracer at the end of synthesis (EOS) (Fig. 4).

For the clinical studies presented here, doses of ^{68}Ga -DOTATATE produced during a single radiolabeling reaction (using the 1110 MBq, 30 mCi generator) were used for PET/CT imaging of 1–3 patients. The new generators (loaded at the manufacturer site with 1110 MBq, 30 mCi of ^{68}Ge) can produce 932–954 MBq (25.1–25.8 mCi) of ^{68}Ga during the first elutions. This activity is sufficient to formulate a dose of 20.4–21.01 mCi of ^{68}Ga -tracer that can be dispensed into 2–3 doses of ^{68}Ga -DOTATATE. Since this generator can be eluted every 4 h, our experience suggests that up to 4 patients per day can be imaged with radiotracer, under the procedures in the present study, without stretching generator limits.

2.3. Quality control and release specifications for the final dose of ^{68}Ga -DOTATATE

The quality control requirements and their specifications as listed in the ^{68}Ga -DOTATATE certificate of analysis (COA) are shown in Table 3. Recently, the Society of Nuclear Medicine and Molecular Imaging (SNMMI) Clinical Trials Network (CTN) published a harmonized release specification document for ^{68}Ga -DOTATOC. The COA analysis of ^{68}Ga -DOTATATE adheres to the recommendations of the SNMMI CTN release criteria, as of the writing of this manuscript.

2.4. Determination of ^{68}Ge breakthrough in clinical doses of ^{68}Ga -DOTATATE

For the production of the clinical doses of ^{68}Ga -DOTATATE, ^{68}Ge breakthrough was monitored over a period of 6 months. The ^{68}Ge content in the final doses of radiotracer was $0.0026\% \pm 0.002\%$. Our IND clinical protocol permits the ^{68}Ge content in the clinical doses to be equal to or less than 0.01% (Fig. 5). The limit of ^{68}Ge breakthrough allowed in US clinical trials of ^{68}Ga -radiotracers is higher than the values recommended by the European Pharmacopeia [27]. As postulated by Velikyan et al, the low limit of ^{68}Ge (0.001%) accepted by European Pharmacopeia could be increased at least 100 times without compromising the patient's safety [28].

2.5. Determination of the ^{68}Ge breakthrough in ^{68}Ga eluate used for research applications

During our initial research studies, we monitored ^{68}Ge content directly in ^{68}Ga eluate over the period of 8 months. The average value of ^{68}Ge breakthrough was 0.0032% during the first 6 months of the use of generator and did not exceed 0.0045% when monitored for an additional 2 months (Fig. 6).

2.6. Pre-concentration of ^{68}Ga eluate by ion exchange extraction

Our observations demonstrate that pre-purification and pre-concentration of ^{68}Ga eluate and post-purification of the final dose are not required prior to release of ^{68}Ga -DOTATATE under our IND protocol. On the other hand, this first step of ^{68}Ga pre-concentration may be

recommended for labeling reactions proceeding in large volumes. The reduction of ^{68}Ga volume may be mandatory for clinical applications using two different generators connected in series or using very small amounts ($<5\text{ }\mu\text{g}$) of precursor for labeling. Thus, we evaluated currently available methods of pre-concentration, with the goal of selecting one method, which could deliver ^{68}Ga with the highest %RCY (Fig. 7). We modified the published protocols to adapt them for use of lower molarity HCl recommended for ITG generators. The percent adsorption of ^{68}Ga eluted from the generator and concentrated using commercially available ion exchange resins was 81% for all of the examined resins. On the other hand, desorption of ^{68}Ga (i.e., its elution from resins) varied widely from 54% to 83%. There were minor differences in elution yield of ^{68}Ga for AG-1X8, Strata-XC and SCX, with the highest desorption yields achieved using the AG-1X8 and Strata SCX column. Overall, our results indicated that all of examined resins can be applied for pre-concentration of ^{68}Ga .

The ^{68}Ge content determined in ^{68}Ga eluates collected for these experiments was on the order of 0.0002%, which represents a 10-fold decrease compared to the concentration of ^{68}Ge in generator-eluate measured without the pre-concentration step. Thus, our results suggest that the pre-concentration step can be used to remove about 84% of ^{68}Ge breakthrough (Fig. 8). We further find the efficacies of all ion-exchangers studied to be comparable.

3. Discussion

The organic-matrix based $^{68}\text{Ge}/^{68}\text{Ga}$ generators produced by ITG GmbH Germany perform consistently and provide a high purity ^{68}Ga that can be directly used for radiopharmaceutical preparation without the need for pre-concentration/purification of the eluted ^{68}Ga . This is likely to be valid for most peptides encountered and is consistent with the acidic conditions required for high efficiency radiolabeling with ^{68}Ga for most chelators currently available commercially. In our studies, ITG GmbH generators delivered consistent and reliable elution profiles with efficiency required for routine doses preparation in clinical studies. The simplified protocol for clinical dose preparation; low level of ^{68}Ge content; and absence of metal contaminants in the eluate as verified by producer, define the ITG $^{68}\text{Ge}/^{68}\text{Ga}$ generator as a competitive alternative to other commercially-available generators (Fig. 9). ITG GmbH introduced recently a GMP-grade generator with extended shelf life of 12 months (or 250 elutions).

4. Materials and methods

^{68}Ga ($t_{1/2} = 68\text{ min}$, β^+ intensity = 89% and EC = 11%) was produced by elution of $^{68}\text{Ge}/^{68}\text{Ga}$ generators (ITG GmbH Germany) using 4 ml of 0.05 M HCl. Three generators loaded with 1110 MBq (30 mCi) of ^{68}Ge and one generator with 370 MBq (10 mCi) of ^{68}Ge were evaluated in these studies. Reagents used in all described reactions and in the final product preparation were ultra-pure trace-metal-free grade. Commercially available GMP-grade DOTATATE (5 mg, Bachem) was compounded into doses of $25 \pm 5\text{ }\mu\text{g}$ peptide dissolved in 100 μL 1 M metals-grade sodium acetate (NaOAc; pH = 5.5; Sigma-Aldrich).

Metals grade ascorbic acid (50 mg/mL, Trace SELECT®, Sigma Aldrich) was used as a free-radical scavenger during dose preparation.

4.1. Elution of the generator

$^{68}\text{Ge}/^{68}\text{Ga}$ generator was eluted manually using 0.05 M HCl (Ultrapure trace-metal-free) by fractional elution (1 mL/fraction) or by collecting the entire 4-ml volume of eluate. The radioactivity content of ^{68}Ga eluted from generator and ^{68}Ge content in the final dose [%] were monitored over a period of 6 months for doses prepared for clinical use. This time period was extended for another two months (total of 8 months) for the generators used for research applications.

4.2. Radiolabeling and quality control of clinical dose of ^{68}Ga -DOTATATE

cGMP-produced DOTATATE ($25 \pm 5 \mu\text{g}$), DOTA-D-Phe¹Tyr³-octerotide, where DOTA is 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid), (Bachem) was dissolved in 100 μL 1 M sodium acetate (NaOAc; pH = 5.5) and reacted with ^{68}Ga (6–24 mCi; 222–888 MBq as $^{68}\text{GaCl}_3$) in 2 ml of fractionated eluate. The reaction mixture was incubated for 10 min at 95 °C in a temperature-controlled heating block. After completion of radiolabeling, 0.4 mL of 1 M phosphate buffer (PBS, pH = 7.5) and 2 mL of sterile water for injection (SWFI) were added to the reaction to adjust osmolality of the clinical dose. The dose of ^{68}Ga -DOTATATE was filtered by 0.22 μm Millipore filter and diluted using 5 ml sterile water for injection (SWFI) to reach a final volume of 10 mL. The final quality control protocol for release included tests to determine osmolality; sterility; radiochemical purity; radionuclide identity; and endotoxin level.

4.2.1. Determination of the radiochemical yield of reactions using iTLC and radio-HPLC—Radiochemical purity of ^{68}Ga -DOTATATE was determined by Instant Thin Layer Paper Chromatography (iTLC; Whatman) using 0.2 M sodium citrate (pH = 5.5) as mobile phase and a BioRadScan as an iTLC reader; RF (free ^{68}Ga) = 0.8–1.0, RF (^{68}Ga peptide) = 0.0–0.3. The radiochemical yield of ^{68}Ga -DOTATATE was confirmed using radio-HPLC (Shimadzu, binary pump; C18 column, 5 μm , 4.6 mm $\times$ 150 mm, Waters) and mobile-phases (deionized-distilled water (ddH₂O) + 0.1% TFA (mobile phase A) and acetonitrile + 0.1% trifluoroacetic acid (TFA) (mobile phase B). The gradient profile (flow rate 1.0 mL/min) was as follows: 0–1 min 10% B; 1–10 min 10%–50% B, 10–12 min 50% B; 12–15 min 50%–10%; 15–20 min 10% B. HPLC injections of the dose were done using auto-sampler (injection volume 50 μL). The retention time of the radiolabeled peptide was monitored using a standard inline gamma-detector (Bio-Rad).

4.2.2. Osmolarity, endotoxin and filter integrity tests—The osmolality of ^{68}Ga -DOTATATE was determined using Osmometer model 3250 (Advance Instruments). The final dose of ^{68}Ga -DOTATATE was examined for the presence of endotoxin using 10 $\times$ dilution of radiotracer and the Endosafe®-PTS™ reader. The integrity of the Millipore filter was confirmed using a standard bubble point test.

4.2.3. Sterility test—The sterility of final dose of radiotracer was determined by addition of 0.1mL of ^{68}Ga -DOTATATE added to thioglycolate medium (FTG) and tryptic soy broth

and incubation for 14 days at temperature of 30–37 °C and 20–25 °C, respectively. Media were monitored on a daily basis. The sterility of the dose was confirmed when there was no visual evidence of growth in the tested media.

4.2.4. ^{68}Ge breakthrough test— ^{68}Ge breakthrough was expressed as a percent of the radioactivity of the parent radionuclide (^{68}Ge) measured at 48 h post elution relative to the radioactivity of daughter radionuclide (^{68}Ga) in the eluate at the time of elution as presented previously [22]. All measurements were performed using a PerkinElmer Gamma Counter (Wizard 1475).

4.2.5. ^{68}Ga radionuclide identity—Radionuclide identity was determined based on the decay properties of the ^{68}Ga ($t_{1/2} = 68\text{min}$). Briefly, the radioactivity of a 20–100 μCi QC-aliquot of ^{68}Ga was measured at 0 min and 10 min post-radiolabeling, using Biodex Atomlab 50 dose calibrator, and compared to theoretical decay of ^{68}Ga . The values recorded within 10% of the known half-life were considered as acceptable for release of the dose.

4.3. Pre-concentration of ^{68}Ga eluate

The pre-concentration of ^{68}Ga was done using ion-exchange resins according to published protocols, which were modified slightly to accommodate the use of lower molarity of HCl [29–34]. To select the most appropriate separation method, we tested several anion-exchange (Bio-Rad AG1-X8, Wax Oasis Waters, Macherey-Nagel 30-PS- HCO_3 Chromafix) and cation-exchange resin (Phenomenex Strata XC, Bio-Rad AG50W-X8) and determined their efficacy for ^{68}Ga pre-concentration.

4.3.1. Pre-concentration of ^{68}Ga eluate using anion-exchange resin

4.3.1.1. Oasis WAX resin: ^{68}Ga eluted from the generator using 4 mL of 0.05 M HCl was mixed with 4 mL of 9.5 M HCl (30%) and loaded on the pre-equilibrated Oasis WAX anion-exchange resin (30 μm , 30 mg). The flow through volume was discarded and the resin was washed with 1 mL of 5 M HCl to remove potential metal contaminants. The purified ^{68}Ga was recovered from the resin soaking and slow washing with 500 μL of ddH₂O [35]. Alternatively, after washing the resin with 1 mL of 5M HCl, two extra rinses were added to decrease the acidity of the final-concentrated fraction of ^{68}Ga . The first rinse of the resin included the use of 1 mL of 5 M NaCl, followed by 1 mL of ethanol, which served to reduce concentration of Cl^- . Finally, the concentrated ^{68}Ga was released from the resin using 0.5 mL of ddH₂O [22].

4.3.1.2. AG-1-X8 resin: The strong anion exchange resin, AG-1-X8 resin (Bio-Rad, 200–400 dry mesh) was pre-condition and activated by washing with 1 mL of 4 M HCl, 1 M HCl, ddH₂O and again 4 M HCl. 4 mL of concentrated 9.5 M HCl (30%) was mixed with $^{68}\text{GaCl}_3$ in 0.05 M HCl (4 mL), in order to form $[\text{}^{68}\text{GaCl}_4]^-$, and loaded together on AG-1-X8 resin. The flow through volume was discarded and the resin was purged with air and washed out manually—first with 1 mL of 5M HCl, followed by 1 mL of 5 M NaCl, and finally 0.5 mL ddH₂O to collect concentrated ^{68}Ga [29,34].

4.3.1.3. 30-PS-HCO₃ Chromafix resin: The 30-PS-HCO₃ anion resin (SPE cartridge Chromafix, Machanery-Nagel, Germany) was pre-equilibrated with 1 mL of 9.5 M HCl (30% HCl), followed by 10 mL of ddH₂O [29,34]. ⁶⁸Ga (5 mL in 0.05 M HCl) was mixed with 4 mL of 30% HCl to reach a final concentration of 5 M HCl. This acidic solution of ⁶⁸Ga was loaded on the 30-PS-HCO₃ anion resin. The cartridge was washed first with 1 mL of 5M HCl, and then two extra rinses were added to decrease acidity of final concentrated fraction ⁶⁸Ga. First rinse of the resin included the use of 1 mL of 5 M NaCl, followed by 1 mL of ethanol. The concentrated ⁶⁸Ga was released from the resin using 0.5 mL of ddH₂O.

4.3.2. Pre-concentration of ⁶⁸Ga eluate using the cation-exchange resin

4.3.2.1. AG50W-X8: 40 mg of the AG50W-X8 resin (Bio-Rad, 200–400 dry mesh size) was loaded into mini-column (1 cm inner diameter). The resin was pre-equilibrated by washing with 1 mL of ddH₂O, followed by 1 mL of 8 M HCl. Concentrated HCl (4 mL) was mixed with Ga⁶⁸ eluate (4 mL) and loaded on the resins, followed by washing with 1 mL saturated 5M NaCl. The pre-concentrated ⁶⁸Ga was collected by final slow elution of resin with 0.5 mL of deionized water [29,30].

4.3.2.2. Strata-XC:

- A.** *A) Method 1:* The Strata XC resin (33 µm, Strong Cation, 30 mg/mL, Phenomenex) was used without preconditioning. The ⁶⁸Ga eluate (4 mL) was loaded on the resin and the flow through volume was discarded. The resin was washed with 80% acetone/0.15M HCl. The concentrated ⁶⁸Ga was desorbed from resin in next step by washing slowly with 0.4 mL of 98% acetone/0.05 M HCl [31,32].
- B.** *B) Method 2:* 4 mL of ⁶⁸Ga in 0.05 M HCl was pre-concentrated by loading manually on the Strata-XC cartridge. The flow through was discarded and the resin was washed directly with 90% acetone/0.02 M HCl to give 500 µL fraction of concentrated ⁶⁸Ga [34].

4.3.2.3. Strata-SCX: Strata-SCX column (Varian, Bond Elut-SCX, 100 mg, 1 mL column) was pre-equilibrated by washing with 1 mL of 5.5 M HCl, followed by 10 mL of ddH₂O. A 20 µL volume of 5.5 M HCl was added to 5 mL of Ga⁶⁸ eluate in 0.05 M HCl and loaded on the Strata-SCX column. The flow through was discarded and the cartridge was washed slowly with 1 mL of 5 M of NaCl to collect concentrated ⁶⁸Ga eluate [34].

5. Contributions

I.T. and D.R. designed and conducted all experiments and have an equal contribution. S.M., K.Z., S.T. provided assistance throughout experimentation. M.S. provided conceptual assistance on ⁶⁸Ga purification strategies. M.S., S.M., K.Z., D.R., S.T., E.D., and A.M. reviewed the manuscript. I.T. analyzed the data and wrote the manuscript, with editorial assistance of M.S.

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Fig. 1.

Fractional elution of ^{68}Ga eluted manually from the GLP-grade ITG GmbH $^{68}\text{Ge}/^{68}\text{Ga}$ generator. Three different generators were evaluated over the period of 5 months (eluted 167 times), 6 months (eluted 158 times) and 9 months (eluted 155 times). Thus each bar represents $n = 480$ experiments. The highest activity of ^{68}Ga is collected in the second and third 1-mL fractions with about 10% radioactivity found in the remaining fractions.

Fig. 2. Stability of ^{68}Ga elution yield determined over the shelf life of the evaluated generators. (a) The radioactivity of ^{68}Ga eluted from the generator (experimental value) was compared to activity of ^{68}Ga expected based on the certified value of loaded ^{68}Ge (decay-corrected) provided by the manufacturer (theoretical). Each data point on the graph represents average yield of ^{68}Ga eluted from the generator for sequential 25 elutions. Each error bar defined standard deviation of these data. (b) The radioactivity of ^{68}Ga eluted during the shelf life of

the generator. Each bar represents average values of ^{68}Ga [mCi] eluted from the generator sequential 25 elutions. The error bar represents variations in these values.

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Fig. 3.
Flow chart showing the radiosynthesis of ^{68}Ga -DOTATATE.

Fig. 4. Radiochemical purity (%RCP) and radiochemical yield (%RCY) of the final of ^{68}Ga -DOTATATE as determined at the time of calibration (TOC) and evaluated over a six month time period (n = 20 repetitions).

Fig. 5. ^{68}Ge breakthrough (%) determined in the clinical doses of ^{68}Ga -DOTATATE using three different generators. Each bar represents the average values ^{68}Ge breakthrough (%) as determined in clinical doses of Ga-DOTATATE prepared during one month. These values of ^{68}Ge breakthrough are also listed in table below the graph. The average value of ^{68}Ge determined in the final dose is 0.0026%.

Fig. 6.

^{68}Ge breakthrough (%) determined directly in ^{68}Ga eluate used for research applications using one generator. Each bar represent the average values ^{68}Ge breakthrough in eluate calculated every month for 8 consecutive months. This generator used for research applications and the average values of ^{68}Ge are shown in table listed below graph (0.00247%).

Fig. 7.

Yield of ^{68}Ga retention and desorption using cation and anion exchange resins ($n = 10$ experiments). Activities of ^{68}Ga retained and desorbed from the resin were compared to the total loaded activity of ^{68}Ga . These values of ^{68}Ga retention and desorption were not decay corrected (all processes were completed within the same time frame).

Fig. 8.
% ^{68}Ge breakthrough in ^{68}Ga eluate before and after its pre-concentration using cation and anion exchange-resins (n = minimum 10 experiments). The data were collected during 8 months of the use of generator.

Fig. 9.
Comparison of protocols used for production of clinical dose of ^{68}Ga -DOTATATE using all three commercially-available generators.

Table 1

Technical specifications of the ITG GmbH $^{68}\text{Ge}/^{68}\text{Ga}$ generator provided by the manufacturer.

Technical specification	ITG $^{68}\text{Ge}/^{68}\text{Ga}$ Generator
Elution	4 ml of 0.05 N HCl
Ge-68 breakthrough	Ge-68 content < 0.005% of activity at the TOC ^a
Elution yield	>80% on calibration date
Shelf life	6 months (GMP prototype); 12 months (GMP certified in 2014)
Radiochemical purity of eluate	>95% Ga^{3+}
Radionuclidic purity of eluate	>99.9% Ga-68
Chemical purity of eluate	No metallic contaminants ^b
Bacterial endotoxins in eluate	<100 EU/ eluate
Sterility	Sterile (GMP-produced)
Generator activity size	370 MBq to 2 GBq

^a TOC: at the time of calibration by ITG GmbH.

^b as determined by ITG GmbH.

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Table 2

Percent of ⁶⁸Ga expected to be eluted relative to the certificate value of total ⁶⁸Ge provided by the manufacturer.

Time (h)	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
Ga-68 activity (%)	45.7	70.6	84.0	91.3	95.3	97.4	98.6	99.2	99.6

Table 3

Quality control specifications for clinical doses of ^{68}Ga -DOTATATE based on the FDA approved protocol (IND 117289).

Test	Required specification	Observed parameters
Appearance	Clear	Clear
Endotoxin	<1 EU/mL	<0.5 EU/ml
pH	5–7.5	PASS
Radionuclide Identification	Decay property corresponding to Half life of ^{68}Ga (68 min)	PASS
Total Radioactivity	4–16 mCi	$7.34 \pm 3.3 \text{ mCi}^a$
Radiochemical purity	>95%	$98.998 \pm 1.1\%^a$
Osmolarity	250–350 mOsmol/kg	PASS
Filter Integrity	Pass (50 psi)	PASS
Volume; injection solution	8.0–9.5 mL	9.1 ± 0.3^a
Sterility ^a (Results after 2 weeks)	Pass (Sterile)	PASS
Germanium Breakthrough ^a (results after 1 days)	<0.01%	$0.00265 \pm 2.0\text{E-}03\%$

^a Average values.