

Validation of Quality Control Parameters of Cassette-Based Gallium-68-DOTA-Tyr3-Octreotate Synthesis

Abstract

Purpose of the Study: Gallium (Ga)-68-DOTA peptides targeting somatostatin receptors have been assessed as a valuable tool in neuroendocrine tumor imaging using positron emission tomography. However, at the moment, a specific monograph in the European Pharmacopoeia (Ph. Eur.) does exist only for Ga-68-dotretotide (DOTATOC) injection. Here, we report on the validation process of Ga-68-DOTA-Tyr3-octreotate (DOTATATE) cassette-based production and quality control (QC). **Materials and Methods:** Preparation of Ga-68-DOTATATE was performed according to the current European Union-good manufacturing practices, the current good radiopharmacy practice, the Ph. Eur., and the guidelines on validation of analytical methods for radiopharmaceuticals. Process was validated via three consecutive production runs to ensure that the methods are reproducible and reliable in routine use. The QC tests for Ga-68-DOTATATE were radiochemical purity (RCP – high-pressure liquid chromatography [HPLC]), radiochemical impurities $^{68}\text{Ga}^{3+}$ (HPLC and instant thin layer chromatography [ITLC]), chemical purity (HPLC and gas chromatography [GC]), pH (pH-strips), radionuclidic purity (principal γ -photon), germanium-breakthrough (^{68}Ge -content), Ga-68 half-life (γ -ray spectrometry), and sterility/endotoxin assay. **Results:** Radiolabeling procedure of Ga-68-DOTATATE fits all the applicable Ph. Eur. specifications. RCP measured via ITLC was >99% in the three validation batches. HPLC-measured RCP resulted 99.45%, 99.78%, and 99.75%. Germanium-breakthrough was far below the recommended level established in the Ph. Eur. Ga-68-DOTATOC injection (#2482). Residual ethanol tested with GC was less than 10%. All the batches were tested for endotoxin content, which always resulted lower than 17.5 EU/ml. All preparations passed the sterility tests. pH of the final product was 7 in all samples. **Conclusion:** Ga-68-DOTATATE fulfilled all the pre-set QCs and release criteria in the batches considered for this validation study. The results demonstrated a batch-to-batch reproducibility, ensuring that synthesis process leads to the expected final product in terms of yield, quality, reliability, safety, and efficacy.

Keywords: Gallium-68, positron emission tomography, radiopharmaceutical validation, somatostatin receptors

Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms that arise from the cells of the endocrine and nervous systems. These tumors can originate from various areas of the body but are most commonly found in the gastrointestinal or bronchopulmonary system.^[1]

Many NETs may be characterized by a spectrum of overexpression of somatostatin receptors (SSTRs) on the cell surface.

SSTRs are G-protein coupled transmembrane receptors that are internalized after binding to specific ligand. Six different SSTRs have been identified:

SSTR1, 2A, 2B, 3, 4, and 5, and the subtypes are co-expressed by the majority of NETs. SSTR2 is the most abundant in the majority of endocrine pancreatic and endocrine gastrointestinal tract tumors, while normal tissue majorly expresses SSTR3 and 5.^[2,3] SSTRs' overexpression represents the ideal target for imaging and therapy of NETs with radiolabeled somatostatin analogs.^[3,4] In-111-labeled octreotide has been considered for many years, the standard diagnostic radiopharmaceutical for imaging SSTRs' expression in tumors using single photon computed tomography (SPECT).

Recent availability of good manufacturing practice Good manufacturing practices

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(GMP)-grade generators for the production of the positron emission tomography (PET) tracer Ga-68 and publication of European Pharmacopoeia (Ph. Eur.) monograph (#2464) on Ga-68-chloride from generators^[5] have changed the imaging scenario. Various new diagnostic agents based on Ga-68 labeling have been added to the clinical activity, due to the use of automated synthesis systems.^[6-8]

In particular, radiolabeling of DOTA-conjugate peptides with Ga-68 allows detecting SSTRs overexpressed on the NET cells providing *in vivo* visualization of primary tumor and metastatic lesions. ⁶⁸Ga-DOTA-peptides (Ga-68-edotreotide[⁶⁸Ga-68-DOTATOC], Ga-68-DOTA-NOC, and DOTA-Tyr3-octreotate [⁶⁸Ga-68-DOTA-TATE]) have different affinity profile for SSTR subtypes: all Ga-68-labeled peptides can bind to SSTR2, while they present different affinity for other SSTRs.^[2] In particular, Ga-68-DOTANOC shows a good affinity for SSTR3 and 5, Ga-68-DOTATOC binds also to SSTR5, and Ga-68-DOTATATE presents a predominant affinity for SSTR2, with an order of magnitude higher than other Ga-68-DOTA-peptides.^[9] Finally, theranostic pairs have been developed labeling the same DOTA-peptide (DOTATATE) with imaging (Ga-68) and therapy (Lu-177) radioisotopes.^[10] The “therapy” probe (Lu-177-DOTATATE, Lutathera[®]) has been approved in Europe (2017), the United States (2018), and Italy (2019) for the treatment of unresectable or metastatic well-differentiated SSTR-positive gastroenteropancreatic NETs in adults.

Since 2013, our radiopharmacy laboratory developed and validated the synthesis and quality control (QC) methods of Ga-68-based radiopharmaceuticals, such as Ga-68-PSMA-HBED-CC for prostate cancer,^[11] Ga-68-PENTIXAFOR for molecular imaging of the CXCR4/CXCL12 axis,^[12] Ga-68-NODAGA-Exendin-4 for imaging of insulinoma, and Ga-68-DOTATOC.

Starting on September 2019, we have set up the synthesis method and QC procedures of the PET probe Ga-68-DOTATATE.

Such radiopharmaceuticals are prepared in our hospital radiopharmacy in accordance with the current European Union-GMP (EU-GMP), the current good radiopharmacy practice,^[13] the Ph. Eur., and the recent European Association of Nuclear Medicine (EANM) guidelines on the validation of analytical methods for radiopharmaceuticals.^[14]

According to these regulations, the manufacturing process has to be validated and quality of the prepared radiopharmaceutical must meet the established acceptance criteria before release,^[13,14] relating to pharmaceutical parameters (bioburden, pyrogenic or particulate contamination, etc.) and radioactivity measures (ensuring high radiochemical purity [RCP] and radionuclidic purity).^[15-17]

However, in the real world of a small-scale radiopharmacy, the lack of peer-reviewed QC guidelines for method

requirements, reference values, and application in human patients has been a major concern for many years, requiring applying general monographs in testing quality of prepared radiopharmaceuticals.

Few PET radiopharmaceuticals are already described in specific monographs in the Ph. Eur. such as Ga-68-DOTATOC injection^[18] or approved in some countries for clinical routine use (such as F-18-FDOPA or F-18-choline).

Recently, the EANM has published general recommendations for the validation of analytical methods to assess the quality of radiopharmaceuticals, improve the management of the quality assurance system in the radiopharmacy, and also reaffirm the need to validate radiopharmaceuticals for human use.^[14]

In this way, acceptance criteria for QC parameters require standardization and validation during the entire process of preparation, demonstrating that the procedure will be reproducibly achieving the desired outcome.^[19-21]

In this manuscript, we report on the validation process of Ga-68-DOTATATE cassette-based production and QC.

Materials and Methods

Reagents

All reagents were high-purity pharmaceutical grade. Hydrochloric acid solution (HCl 0.1 N) was obtained from Rotem GmbH.

The reagents used for high-pressure liquid chromatography (HPLC) and instant thin layer chromatography (ITLC) phase (trifluoroacetic acid [TFA], acetonitrile, and ammonium acetate) were ultrapure or trace metal-free and were purchased from Sigma Aldrich (Saint Louis, Missouri, USA).

The GMP-grade peptide DOTATATE was obtained from ABX pharmaceuticals (ABX, Advanced Biochemical Compounds, Radeberg, Germany) as a 1 mg powder which is dissolved in 1 ml of water and used in 10 µl aliquots.

Automated module and synthesis cassettes

The synthesis of Ga-68-DOTATATE was performed using a fully automated module (Scintomics GRP[®], Fürstfeldbruck, Germany) specifically designed for the synthesis of radiopharmaceuticals.

The module is controlled by a computerized system which runs the valves and syringes on the cassettes producing the desired radiopharmaceutical.

There is a specific cassette for the synthesis of Ga-68-DOTATATE as well as for the generator elution. The production cassettes, sterile and single use, are obtained from ABX pharmaceuticals.

The system is used for the production of other radiopharmaceuticals, such as Ga-68-PSMA,

Ga-68-DOTATOC, and Ga-68-PENTIXAFOR, using a specific synthesis cassette. For each specific radiopharmaceutical process, there is a specific template computer program.

Generator

Ga-68 was obtained from a GMP-grade 68-Ge/68-Ga generator (GalliaPharm®, Eckert and Ziegler, Berlin, Germany).

The available generator contained 1.85 GBq (50 mCi) Ge-68 at calibration date.

In our radiopharmacy routine, elution is performed on the 1st day of the week and on nonsynthesis days, due to the build-up of metal ions on the column requiring to be eluted on a daily basis and within 24 h before any Ga-68-labeled probe synthesis. Elution process is performed using a 0.1 M HCl solution (Rotem GmbH, Germany) driven by the GRP™ Interface software (Scintomics GRP®, Fürstfeldbruck, Germany) in approximately 5 min. The elution volume is 10 ml.

Labeling procedure

Synthesis of Ga-68-labeled [1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic-acid]-Tyr3-octreotate was performed using 20 µg of peptide [Figure 1].

Chemical formula was C₆₅H₉₀N₁₄O₁₉S₂.

The synthesis scheme is shown in Figure 2.

Packaging of the sterile synthesis cassette is removed in a laminar flow hot cell (NMC 68-Ga Tema Sinergie) and all connections are tightened. Then, the cassette is attached to the synthesis module. Subsequently, an empty container, the syringe, the waste bottle, and reactor vials were attached.

The reagents (sodium chloride solution, ethanol, ethanol/water 1/1, water for injection Ph. Eur. and DOTATATE peptide in 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid [HEPES] buffer solution) are placed on the synthesis cassette after closing all valves, and the generator tubes are connected.

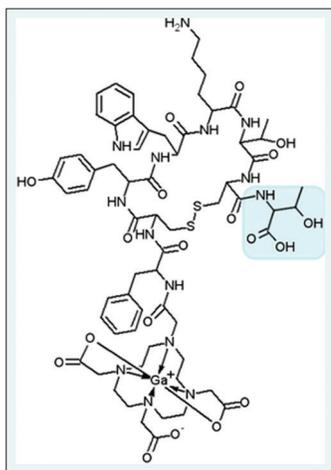


Figure 1: Structure of gallium-68-DOTA-Tyr3-octreotate

List of starting materials used in a Ga-68-DOTATATE batch production are illustrated in Supplementary Table 1.

Ga-68 chloride is eluted from the generator with 10 ml of sterile HCl 0.1 M and collected to the pre-purification exchange cartridge. It is released from the column with 1.6 ml of NaCl 5 M and added to the reaction vial containing DOTATATE peptide (20 µg) in 1.5 M HEPES buffer solution at pH 4.

The mixture is incubated at 95°C for 10 min. After incubation, the content of the reaction vial is pushed through the C₁₈ exchange cartridge (initially conditioned with ethanol, followed by water for injection Ph. Eur.) where the Ga-68-DOTATATE is trapped, while Ga-68 ion and the unbound peptide are sent to the waste. The trapped Ga-68-DOTATATE is recovered from the C₁₈ cartridge by 2 ml of ethanol/water solution (1:1).

The final product is diluted adding 10 ml of phosphate-buffered saline and sterilized by filtration through an inline 0.22 µm sterile filter with a PVDF membrane.

At the end of the synthesis process, a report is saved on the computer for the auditing trail.

Analysis and quality control

Applicable Ph. Eur. specifications for QC of Ga-68-DOTATATE were derived from the monograph of Ga-68-DOTATOC injection.^[18] For validation of typical analytical procedures (e.g., analysis of residual solvents), the ICH guidelines (CPMP/ICH/381/95) have been applied,^[22] while specific adjustments were performed for radioanalytical methods, such as radio-HPLC, radio-TLC, and gamma spectrometry.^[23-30] For the identification test, the “pharmaceutical” part of the radiopharmaceutical was identified comparing the chromatographic retention time of the main radioactive peak and the “cold” standard,^[29] radionuclide

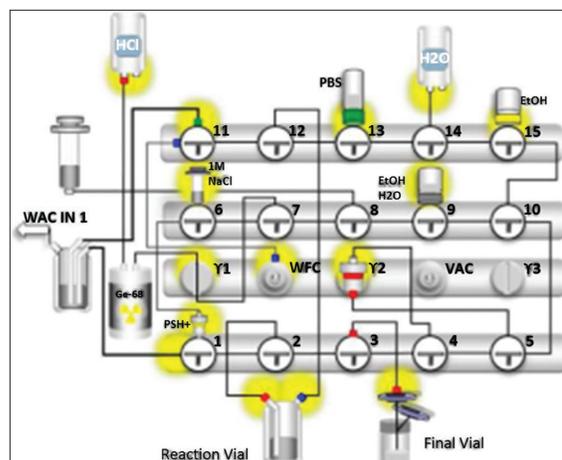


Figure 2: Synthesis scheme of automated Scintomics module GRP® to produce gallium-68-DOTA-Tyr3-octreotate

identification was performed using gamma spectrometric analysis of the emitted energies.^[26]

All the analytical procedures were performed as defined in the standard operating procedures established in our Radiopharmacy Laboratory.

Radiochemical purity

Instant thin layer chromatography

The QC of Ga-68-DOTATATE was performed by ITLC and HPLC, using the 0.2 ml sample taken directly from the final product.

The ITLC test was used to determine the RCP of Ga-68-DOTATATE and to assess Ga-68 impurities, as Ga-68 ion and Ga-68 colloid, in the final product. The ITLC paper strips were counted in the radio-TLC scanner (Cyclone[®] Plus Storage Phosphor system, Perkin Elmer). The percentage of Ga-68 colloid was measured using a 1 mol/L ammonium acetate/methanol (1:1) ITLC-SG strip (Varian ITLC-SG plates): the retention factor (Rf) value was used to identify Ga-68 colloid (Rf = 0.0–0.2) and Ga-68 peptide (Rf = 0.8–1).

High-pressure liquid chromatography

HPLC analyses were performed on a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific) equipped with an Acclaim[™] 120 C18 column 3 μm 120Å (3.0 mm × 150 mm) and a UV and a γ-detector (Berthold Technologies, Milan, Italy). The used solvents were (A) water + 0.1% TFA and (B) acetonitrile + 0.1% TFA. HPLC eluents, water, acetonitrile, and TFA were of high-grade purity. The flow rate of the mobile phase was set at 0.6 ml/min, with a total run of 16 min.

The following gradient was used upon HPLC analysis: 0–8 min 24% B, 8–9 min from 24% B to 60% B, 9–14 min 60% B, 14–14.01 min 60%–24% B, 14.01–16 min from 24% B.

The column temperature was established at 30°C.

The samples were monitored with the UV detector at 220 nm for the detection of chemical impurities, unlabeled peptide, and Ga-68-DOTATATE content in the final product. Ga-68 ion was measured by HPLCγ-detector.

The software system Chromeleon 7 (Thermo Scientific[™] Dionex[™]) was used to put together the information.

To prepare the reference “cold” standard of DOTATATE, we purchased the peptide from Scintomics GmbH. A typical chromatogram of Ga-68 ion is illustrated in Supplementary Figure 1.

Chemical purity

The more plausible chemical impurities in Ga-68-DOTATATE preparation are ethanol and HEPES residuals.

Instant thin layer chromatography

Residual quantity of HEPES was assessed by ITLC, using the 0.2 ml sample taken directly from the final product.

To measure the amount of HEPES, ammonium water/acetonitrile (25:75 V/V) as a mobile phase and ITLC-silica gel strip (ITLC-SG F₂₅₂ plates) as a stationary phase were used. After the chromatographic run, the plate was exposed to iodine vapors for 4 min as required in the monograph of Ph. Eur. ⁶⁸Ga-DOTATOC injection (#2482).^[18] The spot which corresponds to the synthesized radiopharmaceutical must be less intense than the spot of the reference solution (200 μg/V), obtained dissolving 10 mg of HEPES in water (50 ml), volume being the maximum recommended dose in milliliters.

Gas chromatography

Quantitative assessment of any ethanol residues was performed by gas chromatographic (GC) analysis using a sample injection of 1.0 μl, a column of macrogol 20,000 (l = 30 mm; Ø = 0.53 mm; film thickness = 1 μm) as a stationary phase, and helium (He) at a flow of 10 ml/min as a mobile phase.

Temperature was established at 35° C while for injection port was 140° C and 220° C for the detector.

The ethanol identification retention time is in the range of 2–4 min.

The amount of ethanol was calculated from integration peak area of chromatogram and must not exceed 10% V/V or 2.5 g in the final preparation of Ga-68-DOTATATE.

The limit is defined in the European guidelines for residual solvents (EMA/CHMP/ICH/82260/2006),^[24] in the Chapter 5.4 (Residual solvents) of Ph. Eur. 7.0^[31] and in the Ph. Eur. Monograph 68-Ga-DOTATOC injection (#2482).^[18]

Radionuclidic purity

Germanium-68 breakthrough

The Ge-68 breakthrough was determined in an elution sample, after a complete Ga-68 decay (>48 h), on a weekly basis.

The γ-ray spectrometry tests included the identification of principal γ-photon (499–521 KeV peak) and Ge-68 content (decay of 499–521 KeV peak ≥48 h) using a large volume counter linked to a multichannel analyzer system (HPGe detector ORTEC GEM 30P4-76).

Gallium-68 identification and activity measurements

Determination of radionuclidic purity was based on the half-life, the type of emitted radiation, and the energy of the radiation.^[19,26]

Ga-68 was measured with a dose calibrator (Capintec 25-R) in the eluate immediately after elution and in the radiopharmaceutical immediately after preparation.

The γ -ray spectrometric tests included the identification of principal γ -photon (499–521 KeV peak) using a large volume counter linked to a multichannel analyzer system (HPGe detector ORTEC GEM 30P4-76).

Half-life of Ga-68 was calculated after measuring the radioactivity of a sample in the dose calibrator at four consecutive intervals (5, 10, 15, and 20 min), using the equation $t_{1/2} = \ln(1/2)/\lambda$, where λ = decay constant.

pH

The pH value of Ga-68-DOTATATE was measured using pH strips (0–14) which show different colors at different pH range of the samples. The estimated value was registered.

Endotoxin and sterility

Quantitative determination of bacterial endotoxins was performed by the chromogenic method, using an Endosafe[®] Portable Test System (PTS). Ga-68-DOTATATE samples were previously diluted and then applied in duplicate inside cartridges in parallel with positive control testing. The radiopharmaceutical was considered apyrogenic when the level of endotoxins was less than 17.5/V IU/ml.

The sterility of the Ga-68-DOTATATE solution was assessed by direct inoculation of radiopharmaceutical solution in growth broth (Tryptic Soy Broth [TSB]), which was incubated at 20°C–25°C, and verified daily over 14 days, according to the sterility test prescribed in the monograph radiopharmaceuticals preparations (0125). The sample was considered sterile when microbiological growth was absent.

Verification of the aseptic process was performed using the media fill approach repeated biannually. We perform an aseptic manufacturing procedure using a sterile culture medium (TSB), as described above, in place of the radiopharmaceutical product to verify all the production steps to finally generate an aseptic syringe dose. Media fill test is associated to a personal

aseptic qualification of all operators that simulate each step of the process using the culture medium in place of the drug solution. The microbiological quality of workstations and environment was checked before production of the validation runs.

Results

The synthesis unit with labeled components is detailed in Figure 2.

A total of three radiosynthesis runs were analyzed and the results are summarized in Table 1.

Properties of the acceptable product included a pH of 7, a clear colorless solution free of particles, residual ethanol <10% by volume, and endotoxins <17.5 EU/ml.

The total automated synthesis time was 33 min.

The final product Ga-68-DOTATATE was diluted with 10 ml PBS to reach a final volume of 16 ml. Composition of the injectable solution of Ga-68-DOTATATE is illustrated in Supplementary Table 2.

Appearance

The solution was visually analyzed per batch. The visual inspection of in-house prepared radiopharmaceuticals is necessary before injection to the patient being a measure of process performance and validation.^[32] All the samples were colorless, and no particulate was revealed in any of them

Radionuclide identification

The spectrum obtained by gamma spectroscopy showed peaks at energies equal to 0.511 MeV and 1.077 corresponding to Ga-68.

It was also confirmed by the calculation of the half-life of Ga-68 with the formula:

$$t_{1/2} = 0.693 t / (\ln A_0 / A_t)$$

Approximate half-life of Ga-68 was 67.71 min.

Table 1: Quality control results of [⁶⁸Ga]-DOTA-Tyr³-octreotate synthesis

QC data	Run 1	Run 1	Run 3
Appearance	Colorless, no particles	Colorless, no particles	Colorless, no particles
Radionuclidic identity (min)	67.71	67.71	67.71
Radionuclidic purity	100.00%	100.00%	100.00%
Radiochemical identity	RT=5.508 min	RT=5.510 min	RT=5.506 min
Radiochemical purity	99.45%	99.78%	99.75%
Chemical purity	Compliant	Compliant	Compliant
⁶⁸ Ge impurity	$4.9 \times 10^{-5}\%$	$4.7 \times 10^{-5}\%$	$4.8 \times 10^{-5}\%$
pH	7	7	7
LAL test (EU/ml)	<17.5	<17.5	<17.5
Residual EtOH content (% V/V)	5.32	5.48	5.45
Yield (%)	63.34	63.71	64.01
Activity (MBq)	463	459	464

QC: Quality control, RT: Radioreceptor therapy, LAL: Limulus ameobocyte lysate

Germanium-68 breakthrough

The Ge-68 breakthrough determined in the three samples was constantly far below the level recommended by the Ph. Eur. (0.001% of the total radioactivity) and, respectively, $4.9 \times 10^{-5}\%$, $4.7 \times 10^{-5}\%$, and $4.8 \times 10^{-5}\%$.

Radiochemical quality control

RCP as determined by HPLC in the three consecutive runs was 99.45%, 99.78%, and 99.75% respectively. Under the chromatographic conditions described above, Ga-68-DOTATATE peak was well resolved; the average retention time was 5.5 min, as illustrated in typical chromatogram [Figure 3], compared to retention time of cold DOTATATE standard solution. The typical chromatograms of blank eluent and cold DOTATATE are illustrated in Supplementary Figure 2. The RCP was also confirmed with ITLC which showed only the presence of Ga-68-DOTATATE at a retention time of 0.8–1 [Figure 4] in all batches.

Chemical purity

Measured chemical impurities are residual ethanol (verified by GC) and HEPES (verified by ITLC). As shown in the GC [Figure 5], the peak related to the presence of ethanol is at a retention time of 2.10 min. The amount of ethanol was <10% in all batches (respectively 5.32%, 5.48%, and 5.45% V/V).

The residual quantity of HEPES in the final preparation was less intense than the spot corresponding to the reference solution according to the Ph. Eur. (200 µg/V).

pH and sterility

The pH was 7 in all the validation runs and bacterial endotoxins resulted < 17.5 EU/ml in all the three synthesis samples (respectively, 5.00, 4.95, 4.98 EU/ml and 85%,

84.15%, 84.66% for the spike recovery). Microbiological analyses of the media fill tests carried out on all vials obtained at the end of each step showed no microbial growth. All media fill test of the environment and operators resulted were negative.

Reproducibility

The activity of the final product (Ga-68-labeled DOTATATE) depends on the Ga-68 activity eluted from the Ge-68/Ga-68 generator. Starting from an eluate (⁶⁸GaCl₃) of 790 ± 10 MBq produced by our generator, the automated synthesis module can produce 430–460 MBq of Ga-68-DOTATATE.

The batches produced for the validation process contained 463, 459, and 464 MBq of Ga-68.

The radiochemical yield of the validation runs was 63.34%, 63.71%, and 64.01%. The RCP estimated by radio-HPLC resulted 99.45%, 99.78%, and 99.75%.

The total production time of Ga 68 labeled peptide was ~45 min including the synthesis process of 33min and 15 min for all QC testing necessary for the release.

Discussion and Conclusion

Validation of the procedures used in the radiopharmacy laboratory is the mean to assure that prepared radiolabeled probes will be of the required quality.

The continuing development of new tools for molecular imaging is frequently not accompanied by a coherent effort in development, standardization, and validation of QC methods to guarantee high-quality radiopharmaceutical production, especially in the routine clinical setting, requiring more often the use of targeted imaging probes.

During years, we have set up some SPECT and PET tracers for research or clinical purpose,^[11,12,33-35] therefore, it was

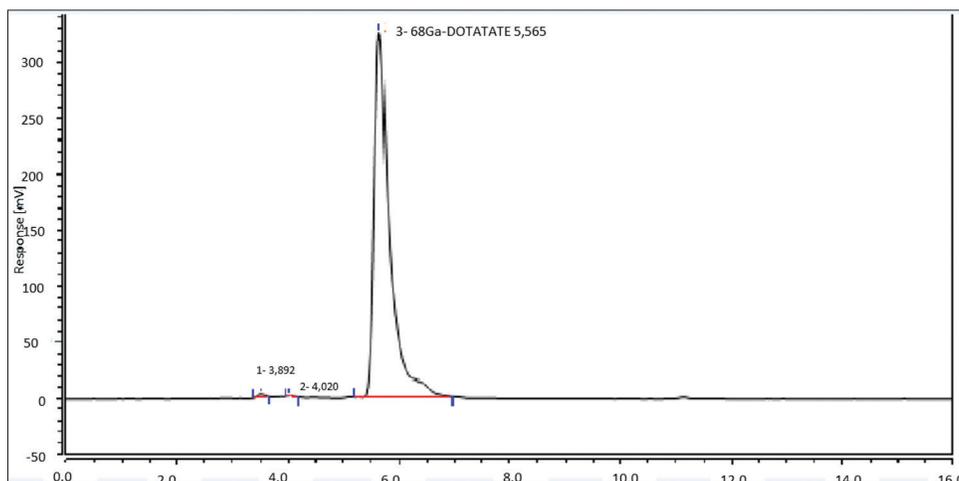


Figure 3: High-pressure liquid chromatogram of gallium-68-DOTA-Tyr3-octreotate. The peaks seen in these two chromatograms were analyzed in UV channel; therefore, only the solvent signal (0.0–2.0 min) is seen, the DOTA-Tyr3-octreotate signal (5.5 min) and no free gallium-68. The peak seen in both chromatograms (10–12 min) is not related to a substance or free gallium-68, but it is related to the gradient high-pressure liquid chromatography method (used to analyze the sample). The labeling efficiency of the three validation runs was 63.34%, 63.71%, and 64.01%

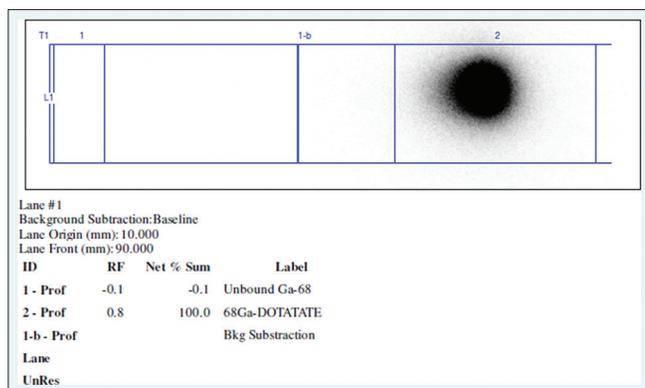


Figure 4: Instant thin layer chromatography of gallium-68-DOTA-Tyr3-octreotate

mandatory to define a validation master plan (EU-GMP, annex 15), covering all the intended validation/qualification activities for radiopharmaceutical preparations, according to the existing guidelines.

Recent commercial automated synthesis systems based on disposable cassettes for each radiolabeled probe simplified the production of multiple tracers avoiding the need of tracer-specific customization. The major advantage of automated system over manual synthesis is the increased reproducibility by reducing operator variability.^[8,36]

However, these systems enable the operator to develop and optimize synthesis protocols of radiopharmaceuticals to achieve the desired yield and purity. Then, a validation of the synthesis process has to be performed for the production of clinical-grade tracers to assess high-quality production and reproducibility.

Moreover, in the clinical context, hospital-based radiopharmacy has to comply the GMP regulation (EU-GMP, annex 3)^[17] and the specific guidelines for small-scale preparations at nuclear medicine departments and PET centers.^[14]

High quality of the produced radiopharmaceuticals appears even more critical in the theranostic arena (as in the case of Lu-177-DOTATATE), requiring the greatest accuracy in detecting lesions that will be targeted by peptide radioreceptor therapy.

Finally, validation of the produced radiopharmaceuticals guarantees maintenance of high specific activity requirements for accurate PET tracer quantification.

Our validation runs of Ga-68-DOTATATE have demonstrated highly reproducible synthesis yields (mean value 63.66%).

The RCP measured using both ITLC and HPLC techniques was above 99% in all the validation batches (recommended level 95%).

All the results confirmed a radionuclidic purity better than the required value of Ph. Eur. 0.001% (with a mean value

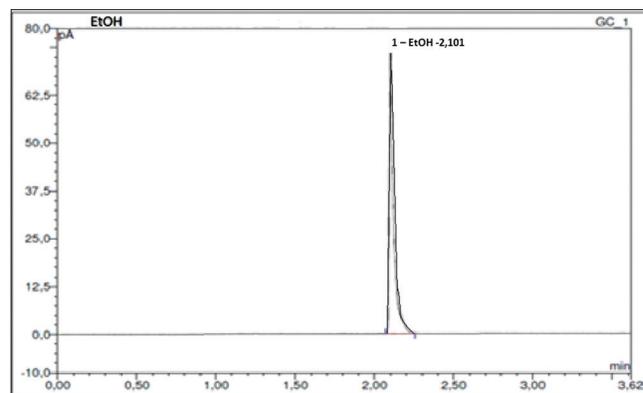


Figure 5: Gas chromatogram of residual ethanol in gallium-68-DOTA-Tyr3-octreotate sample

of $4.8 \times 10^{-5}\%$). Residual ethanol was <10% (mean value 5.42% V/V) and residual HEPES less 200 $\mu\text{g}/\text{V}$ in all batches.

Aseptic manipulations were verified via media fill procedure for synthesis, operators, and environment. Application of media fill tests allowed both to validate operative modality used for Ga-68-DOTATATE synthesis and to attest the ability of operators who worked on it.

Maintenance of high RCP in all the batches associated to the results of facility QCs demonstrated a robust global quality system that guarantees patient safety, image quality, and accurate quantification of tracer accumulation. In addition, a correct and rigorous quality system of radiopharmaceutical preparations allows infection control and prevention, becoming a central objective of daily practice in the nuclear medicine centers.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Hauso O, Gustafsson BI, Kidd M, Waldum HL, Drozdov I, Chan AK, *et al.* Neuroendocrine tumor epidemiology: Contrasting Norway and North America. *Cancer* 2008;113:2655-64.
2. Cescato R, Schulz S, Waser B, Eltschinger V, Rivier JE, Wester HJ, *et al.* Internalization of SST2, SST3, and SST5 receptors: Effects of somatostatin agonists and antagonists. *J Nucl Med* 2006;47:502-11.
3. Reubi JC, Waser B, Schaer JC, Laissue JA. Somatostatin receptor SST1-SST5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med* 2001;28:836-46.
4. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev* 2003;24:389-427.
5. Gallium (68 Ga) chloride solution for radiolabelling. European Directorate for the quality of medicines. *Eur Pharmacopeia* 2013;2464:1060-1.
6. Li X, Yu W, Wollenweber T, Lu X, Wei Y, Beitzke D, *et al.*

- [(⁶⁸Ga) Pentixafor PET/MR imaging of chemokine receptor 4 expression in the human carotid artery. *Eur J Nucl Med Mol Imaging* 2019;46:1616-25.
7. Luo Y, Pan Q, Yao S, Yu M, Wu W, Xue H, *et al.* Glucagon-like peptide-1 receptor PET/CT with ⁶⁸Ga-NOTA-Exendin-4 for detecting localized insulinoma: A prospective cohort study. *J Nucl Med* 2016;57:715-20.
 8. Aslani A, Snowdon GM, Bailey DL, Schembri GP, Bailey EA, Roach PJ. Gallium-68 DOTATATE production with automated PET radiopharmaceutical synthesis system: A three year experience. *Asia Ocean J Nucl Med Biol* 2014;2:75-86.
 9. Reubi JC, Schär JC, Waser B, Wenger S, Heppeler A, Schmitt JS, *et al.* Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur J Nucl Med* 2000;27:273-82.
 10. De Decker M, Turner JH. Automated module radiolabeling of peptides and antibodies with gallium-68, lutetium-177 and iodine-131. *Cancer Biother Radiopharm* 2012;27:72-6.
 11. Migliari S, Sammartano A, Scarlattei M, Serreli G, Ghetti C, Cidda C, *et al.* Development and validation of a high-pressure liquid chromatography method for the determination of chemical purity and radiochemical purity of a [⁶⁸Ga]-labeled Glu-Urea-Lys (Ahx)-HBED-CC (positron emission tomography) tracer. *ACS Omega* 2017;2:7120-6.
 12. Sammartano A, Migliari S, Scarlattei M, Baldari G, Ruffini L. Synthesis, validation and quality controls of [⁶⁸Ga] DOTA Pentixafor for PET imaging of chemokine receptor CXCR4 expression. *Acta Bio Medica Atenei Parmensis*. 4/2020; 9; doi: 10.23750/abm.v9i14.9106.
 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.
 14. Gillings N, Todde S, Behe M, Decristoforo C, Elsinga P, Ferrari V, *et al.* EANM guideline on the validation of analytical methods for radiopharmaceuticals. *EJNMMI Radiopharm Chem* 2020;5:7.
 15. IAEA TECDOC, Quality control in the production of Radiopharmaceuticals. Int Atomic Energy Agency; IAEA-TECDOC-1856 978-92-0-107918-3.
 16. By Kung-Tien Liu, Jian-Hua Zhao, Lee-Chung Men and Chien-Hsin Chen. Quality by design and risk assessment for radiopharmaceutical manufacturing and clinical imaging. Latest Research into Quality Control Chapter 13. IntechOpen, 2012, 255-92.
 17. Annex 3. EudraLex, Manufacture of Radiopharmaceuticals, The Rules Governing Medicinal Products in the European Union Volume 4, EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. European Commission Enterprise and Industry Directorate-General; 2008.
 18. European Pharmacopoeia (01/2013:2482 Gallium (⁶⁸Ga) Edotreotide injection). *Eur Pharm* 2011;23:310-3.
 19. Decristoforo C, Penuelas I, Patt M, Todde S. European regulations for the introduction of novel radiopharmaceuticals in the clinical setting. *Q J Nucl Med Mol Imaging* 2017;61:135-44.
 20. Quality assurance for radioactive measurement in nuclear medicine. IAEA International Atomic Energy Agency 2006, Technical Reports Series. No. 454. 2006.
 21. Todde S, Peitl PK, Elsinga P, Koziorowski J, Ferrari V, Ocak EM, *et al.* Guidance on validation and qualification of processes and operations involving radiopharmaceuticals. *EJNMMI Radiopharm Chem* 2017;2:8.
 22. CPMP/ICH/381/95-ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf. [Last accessed on 2020 Jun 04].
 23. Annex 15 (EU) of Good Manufacturing Practice (GMP) Guidelines: EDQM Guidelines: Guide for the Elaboration of Monographs on Radiopharmaceutical Preparations. European Pharmacopoeia 9th Edition.; 2018.
 24. EMA/CHMP/ICH/82260/2006 ICH Guideline Q3C (R6) on Impurities: Guideline for Residual Solvents. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-33.pdf. [Last update on 2019 Oct 08; Last accessed on 2020 Jun 04].
 25. Decristoforo C, Knopp R, von Guggenberg E, Rupprich M, Dreger T, Hess A, *et al.* A fully automated synthesis for the preparation of Ga-68-labelled peptides. *Nucl Med Commun* 2007;28:870-5.
 26. Detection and Measurement of Radioactivity. European Pharmacopoeia. 9th ed., Vol. 1. Ch. 2.2.66:2017.
 27. ICH Topic Q3C (R4) Impurities: Guideline for Residual Solvents February 2009 CPMP/ICH/283/95 European Medicines Agency; 2010.
 28. General Monograph; Radiopharmaceutical Preparations. (0125). European Pharmacopoeia. 9th ed., Vol. 1. Ch. 5; 2017.
 29. Chromatographic separation techniques. European Pharmacopoeia. 9th ed., Vol. 1. Ch. 2.2.46; 2017.
 30. de Blois E, de Zanger RM, Chan HS, Konijnenberg M, Breeman WA. Radiochemical and analytical aspects of inter-institutional quality control measurements on radiopharmaceuticals. *EJNMMI Radiopharm Chem* 2019;4:3.
 31. Residual Solvents. European Pharmacopoeia 7.0. Chapter 5.4. (50400) 01/2008.
 32. Langille SE. Particulate matter in injectable drug products. *PDA J Pharm Sci Technol* 2013;67:186-200.
 33. Migliari S, Sammartano A, Cidda C, Baldari G, Scarlattei M, Serreli G, *et al.* Novel approach for quality assessment and improving diagnostic accuracy in cell-based infection imaging using ^{99m}Tc-HMPAO labeled leukocytes. *Acta Biomed* 2018;89:355-64.
 34. Sammartano A, Scarlattei M, Migliari S, Baldari G, Ruffini L. Validation of *in vitro* labeling method for human use of heat-damage red blood cells to detect splenic tissue and hemocateretic function. *Acta Bio Med* 2019;90:275-80.
 35. Sartori A, Bianchini F, Migliari S, Burreddu P, Vacondio F, Arosio D, *et al.* Synthesis and preclinical evaluation of a novel, selective ¹¹¹In-labelled aminoproline-RGD-peptide for non-invasive melanoma tumor imaging. *Med Chem Commun* 2015;6:2175-83.
 36. 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 Labelled Compounds Radiopharmaceuticals* 2014;57:615-20.

Supplementary Table 1: List of starting materials used in a gallium-68-DOTA-Tyr3-octreotate batch production

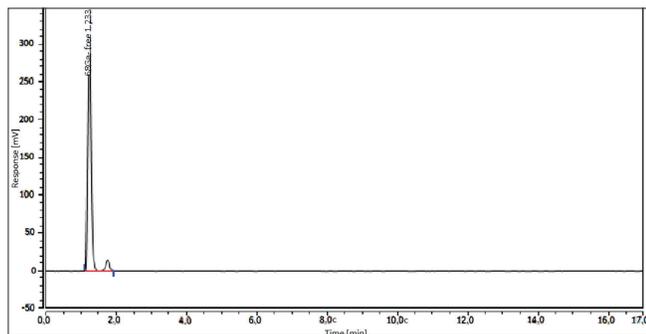
Starting materials	Amounts
DOTATATE	20 µg
Water for injection Ph. Eur.	100±1 ml
5 M NaCl	1.7±0.1 ml
Ethanol Ph. Eur.	5.0±0.2 ml
Ethanol/water 1/1	2.0±0.1 ml
PBS	20±0.5 ml
1.5 M HEPES buffer solution	3.0±0.2 ml
Sterile HCl 0.1 N	10 ml

DOTATATE: DOTA-Tyr3-octreotate, PBS: Phosphate-buffered saline, HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid, HCl: Hydrochloric acid solution, Ph. Eur.: European Pharmacopoeia

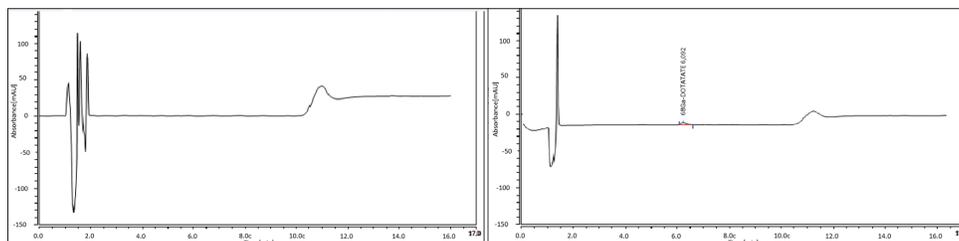
Supplementary Table 2: Composition of the injectable solution of gallium-68-DOTA-Tyr3-octreotate

Component	Quantity	Function
Ga-68-DOTATATE	28.6-29 MBq/ml	Active substance
PBS	10 ml	Excipient
Ethanol/water solution 1/1	2 ml	Excipient
Water for injection	4 ml	Excipient

DOTATATE: DOTA-Tyr3-octreotate, PBS: Phosphate-buffered saline, Ga: Gallium



Supplementary Figure 1: Typical chromatogram of gallium-68 ion



Supplementary Figure 2: High-pressure liquid chromatograms of blank sample (a) and DOTA-Tyr3-octreotate (b)