Chapter

Gallium-68: Radiolabeling of Radiopharmaceuticals for PET Imaging - A Lot to Consider

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Abstract

Gallium-68 was applied for positron emission tomography (PET) imaging already in the early beginnings of PET imaging. Today, with the introduction of PSMA-targeting tracers (e.g. PSMA-11, PSMA-617, and PSMA-I&T), the number of clinical applications of ^{68}Ga-radiopharmaceuticals for diagnostic imaging has grown considerably. This development was initiated and supported already in the mid-2000s by the commercial availability of ^{68}Ge/^{68}Ga generators designed for clinical usage. This progression was accompanied by the development of several purification methods to generator eluate as well as sophisticated ^{68}Ga-radiopharmaceuticals. Due to the ^{68}Ga-rush, the need for implementation of gallium-68 (depending on production route) and its certain tracers into the pharmacopeia increased. Based on the specifications given by the pharmacopeia, interest focused on the development of automated synthesis systems, ^{99m}Tc-analog kits with regard to patient as well as operator safety.

Keywords: gallium-68, radiopharmaceuticals, production, quality control, clinical use

1. Introduction

In recent years, ^{68}Ga-radiopharmaceuticals gained more and more attention due to their steadily growing clinical application. Facilitated is this development by increasing interest in the application of its "theranostic twin" lutetium-177. Combining both, gallium-68 and lutetium-177, enables diagnostic molecular imaging followed by personalized treatment based on the diagnostic scan [1].

This concept is well established for treatment of neuroendocrine tumors (NETs) using peptide receptor radionuclide therapy (PRRT). This approach allows the targeted treatment of inoperable or metastatic NETs already proven in multiple clinical trials employing radiolabeled somatostatin analogs [2–9]. Based on the data received, the U.S. Food and Drug Administration (FDA) recently approved ^{177}Lu-labeled DOTA-TATE for PRRT treatment. However, not only for NETs, but also for other types of cancer (e.g. prostate cancer (PC)), lutetium-177 is of interest, reflected in numerous clinical trials registered at https://clinicaltrials.gov (keyword: lutetium-177; 87 trials; 12/9/2019). Even more trials are enrolled for its diagnostic counterpart gallium-68 (keyword: gallium-68; 268 trials; 12/9/2019). While only a handful clinical trials were conducted before 2012 for both radionuclides

(gallium-68, 12 trials between 1991 and 2011; lutetium-177, 16 trials between 1996 and 2011) both have increasingly found application in clinical routine reflected in the rapidly increasing amount of enrolled phase 1–3 studies.

Although, gallium-68 was already proposed for medical use by Gleason [10] its way to clinical application was not possible without the advancement of the primary generator design. Providing [^{68}Ga]$GaCl_3$ and containing only trace levels of the long-living mother radionuclide germanium-68 regarding ^{68}Ga-activity, the commercially availability of generator simplified research and motivated developments with a view to a broad routine application. The launch of this new type of ^{68}Ga-generator together with decades of research in chelation chemistry and drug discovery resulted in the design of ^{68}Ga-radiopharmaceuticals of high affinity/selectivity for their biological targets [11–13].

The advantages of the generator availability and the easy one-step chelation chemistry ensured the relatively fast and broad application of the ^{68}Ga-radiopharmaceuticals even in smaller institutions. However, exactly these advantages lead to problems in the supply today and require new developments in order to meet the growing demands.

2. Application: why choosing a radiometal?

What is the advantage of radiometals for an application in nuclear medicine? With carbon-11 and mostly fluor-18, two radionuclides for positron emission tomography (PET) are available, which can be used for radiolabeling without appreciably altering the biological properties of the compounds in addition to their favorable decay characteristics. However, the disadvantage of radiometals, the need for a chelator is also their advantage over fluor-18 and carbon-11.

Due to this, radiolabeling with radiometals is very easy, can be conducted in aqueous solution and with the right choice of chelator possible under mild conditions. That enables radiolabeling of temperature or organic solvent sensitive compounds (e.g. antibodies). Additionally, the choice of chelator provides the possibility of radiolabeling one compound with different radiometals. Thus,

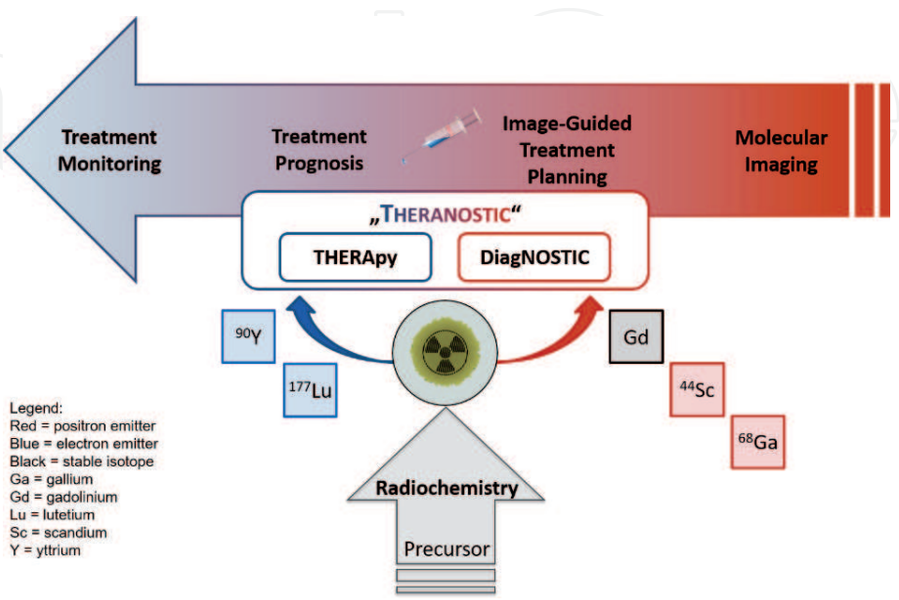

Figure 1.
Depiction of the theranostic concept: utilizing one compound for a variety of applications in patient-centered care radiolabeled with different radionuclide.

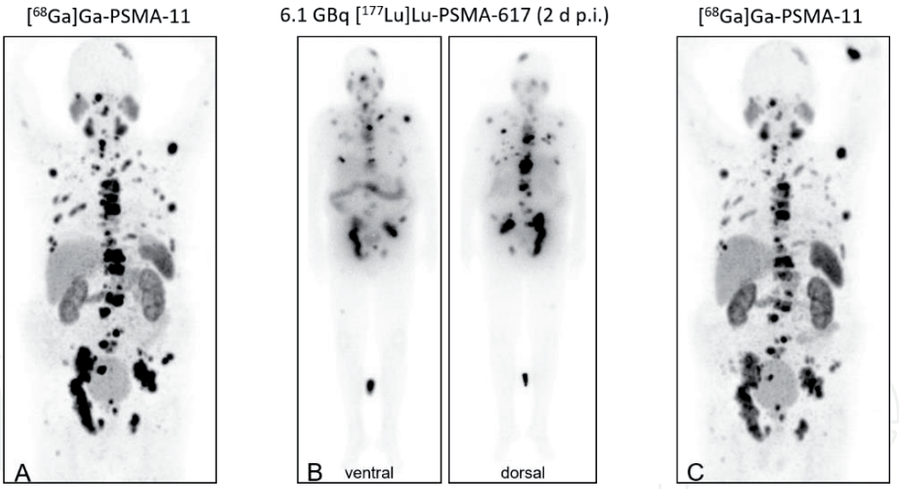

Figure 2.
PET-images (A; C) and SPECT-images (B) of a patient with metastatic castrate-resistant prostate cancer (mCRPC) undergoing therapy with [^{177}Lu]Lu-PSMA-617 with pre- and posttherapeutic ^{68}Ga-PET-imaging using the diagnostic counterpart [^{68}Ga]Ga-PSMA-11.

widespread application (PET, single photon emission computed tomography (SPECT), magnet resonance tomography (MRT) and therapy) of the compound only by exchange of the radiometal with minimum changes in biological behavior is possible. This facilitates patient-centered care from diagnosis via molecular imaging, over treatment planning, prognosis and monitoring utilizing one compound (**Figure 1**).

Advantages in favor of gallium-68 compared with other appropriate radiometals are its favorable decay characteristics, its (commercial) availability and the possible combination with lutetium-177 as theranostic pair (**Figure 2**). Also gallium-68 possibly provides patient care in places where cyclotron-produced fluor-18 is not obtainable.

3. Current applications of ^{68}Ga-radiopharmaceuticals

Currently gallium-68 is most widely used in the diagnosis of prostate cancer in the form of [^{68}Ga]Ga-PSMA-11, respectively. [^{68}Ga]Ga-PSMA-617 together with [^{177}Lu]Lu-DOTA-PSMA-617 forms a theranostic couple, which is very well suited for the diagnosis or treatment of prostate cancer as the ^{68}Ga/^{177}Lu-radiolabelled tracers show a very similar biological behavior. Due to similarities in chemical behavior, identical (in case of PSMA-617) precursors can be radiolabelled using the same or similar equipment, synthesis and quality control methods [14].

The second, but longest known and best evaluated, ^{68}Ga theranostic pair is used for neuroendocrine tumors in combination with various somatostatin analogs. The three most widely used analogs of somatostatin with gallium-68 are [^{68}Ga]Ga-DOTA-TOC, [^{68}Ga]Ga-DOTA-TATE, [^{68}Ga]Ga-DOTA-LAN or [^{68}Ga]Ga-DOTA-NOC [15]. As a therapeutic counterpart, yttrium-90 and lutetium-177 are used.

Besides these two main applications of gallium-68, a variety of studies work on the extension of the application scope.

For imaging of insulinoma pancreatic islets, several versions of ^{68}Ga-radiopharmaceuticals based on Exendin-4, a glucagon-like protein-1 receptor agonist, exist and it was demonstrated that [^{68}Ga]Ga-DOTA-exendin-4 localizes insulinoma significantly better than ^{111}In-radiolabelled radiopharmaceuticals [16].

Integrin αvβ3 and gastrin-releasing peptide receptor (GRPR) are usually overexpressed in human breast cancer, prostate cancer, breast cancer, colorectal cancer, pancreatic cancer, glioma, lung cancer, ovarian cancers, endometrial

cancers, renal cell cancer and gastrointestinal stromal tumors. An amphibian homolog of the mammalian gastrin-releasing peptide bombesin was intensively investigated, also radiolabelled with gallium-68, for imaging of GRPR. For integrin αvβ3, specific imaging probes usually use the peptide arginine-glycine-aspartic acid (RGD). For imagine of GRPR, several radiopharmaceuticals based on gallium-68 were proposed, in particular [^{68}Ga]Ga-BBN-RGD for breast cancer imagine [17], [^{68}Ga]Ga-NOTA-Aca-BBN for glioma imagine [18], [^{68}Ga]-NOTA-DUPA-RM26 for prostate cancer imagine.

Another promising area of application of ^{68}Ga-based radiopharmaceuticals is the labeling of human epidermal growth factor receptor family (HER2) [19] and carcinoembryonic antigen (CEA) [20].

Even though gallium-68 is a very convenient radionuclide for use in radiopharmacy, it is widespread in radiopharmaceuticals in comparison with other diagnostic isotopes. But usability and the commercially availability of generator simplified research and motivated developments with a view to a broad routine application.

4. Radiometals: special needs?

Radiolabeling with radiometals is in some ways challenging. Due to the very low amount of substance, other metals present in the reaction mixture can be serious problem and noticeably effect the radiolabeling. These metallic impurities can compete with gallium-68 for the chelating function of the precursor and are compared with gallium-68 (1 GBq equals to 9.73×10^{-12} mol) even when present at low levels (<ppm) clearly in excess number. They are result of external influences (e.g. production of starting materials) or are an intrinsic generator property (e.g. matrix; decay product). To avoid additional or larger impurities than necessary, the following is recommended by the IAEA [21]:

- Use plastic disposables/contact materials
- Avoid contact with metals of your working equipment during preparation of reagents (e.g. pipettes, spatulas, vials, etc.)
- Protect your working materials from direct contact with metals (e.g. surfaces, etc.)
- Use chemicals and water with lowest metal content as possible (e.g. ultra-pure grade)
- Do not use standard laboratory glassware (e.g. beakers, etc.)
- Consider coating of your fume hood.

5. Gallium-68: a brief profile

Gallium is located in group 13 in the 4th period. It has 31 known isotopes and 11 metastable isomers including the two natural occurring stable isotopes gallium-69 (60.11%) and gallium-71 (39.89%). Two gallium isotopes are applied in nuclear medicine for PET-imaging: gallium-67, which has the longest half-life ($T_{1/2}$ = 3.26 d) of the instable ^{68}Ga-isotopes, and gallium-68 ($T_{1/2}$ = 67.71 min).

Positron emitter	Half-life	$\widetilde{E}_{\beta}$	$E_{\beta, max}$
		[MeV]	
Gallium-68	67.71 min	0.829	1.899
Flourine-18	109.77 min	0.250	0.634

Table 1.
Comparison of mean ($\widetilde{E}_{\beta}$) and maximum ($E_{\beta, max}$) positron energies of gallium-68 and fluorine-18 [24].

Ga(III) is a hard Lewis acid forming complexes coordinating four, five or six ligands. The most stable complexes are the last-mentioned with a octahedral coordination sphere in which oxygen, nitrogen and sulfur donor atoms form coordination bonds with Ga(III). To ensure the complex formation thorough pH, control is required to ensure deprotonation of the electron donor and to protect Ga(III) from forming $Ga(OH)_3$ precipitating at pH 3–7 [22].

Gallium-68 is a positron emitter that decays with a half-life of 67.71 min and 89% positron branching to stable zinc-68. The transition is accompanied by low-abundant photon emission (1077 keV, 3.22%) [23]. **Table 1** shows the mean and maximum energies of the positrons emitted in comparison to fluorine-18.

6. Availability: sources of gallium-68

6.1 Traditional: ^{68}Ge/^{68}Ga-generator

One of the reasons of the emerging application of gallium-68 in nuclear medicine is its cyclotron-independency and availability via radionuclide generator. Since the application of gallium-68 was a long time limited to research, advancements in generator design facilitated research on new ^{68}Ga-radiopharmaceuticals as well as clinical use of the known.

Physical basis for radionuclide generators is the existence of the radioactive equilibria. The differentiation between radionuclide generations is based on the half-lives of the parent (1) and its daughter (2). Depending on the ratio between the two half-lives, three principal cases can be distinguished:

1. Transient equilibrium. Longer living parent but not more than factor 100: $T_{1/2,\,2} < T_{1/2,1} < 100$.

2. Secular equilibrium. Much longer living parent: $T_{1/2,\,2} << T_{1/2,1}$.

3. No equilibrium. Shorter living parent.

The basis for the ^{68}Ge/^{68}Ga-generator is the secular equilibrium between the parent radionuclide germanium-68 and its daughter gallium-68. Germanium-68 decays with $T_{1/2}$ = 270.95 days via electron capture to gallium-68. This transition is subsequently followed by decay of gallium-68 to stable zinc-68. At equilibrium, the quantity of gallium-68 produced is equal to the quantity of gallium-68 decaying, while the parent activity does not significantly decrease over many half-lives of the daughter. The theoretical maximum activity or equilibrium state for a certain generator system can be obtained at the time t (**Figure 3**):

$$t = \frac{1}{\lambda_2 - \lambda_1} ln \frac{\lambda_2}{\lambda_1} \quad (1)$$

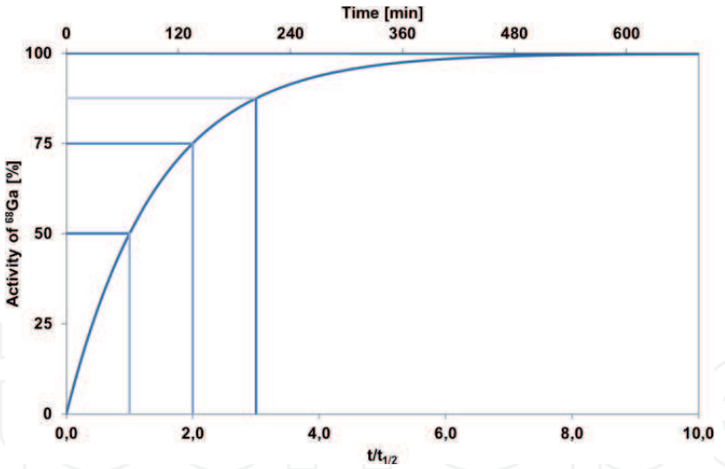

Figure 3.
Build-up kinetics of gallium-68 on the generator column after initial elution.

For the ^{68}Ge/^{68}Ga system, equilibrium is reached after 14.1 h, representing maximum obtainable activity. Even if idle times of 12.5 half-lives are necessary to obtain maximum activities, the generators can be used more frequently. Within two half-lives of gallium-68 already 75% of the maximum value is build-up and could be used.

The ^{68}Ge/^{68}Ga-generator system introduced in the 1960s by Gleason [10] underwent a lot of changes until today. From the first gallium cow providing gallium-68 after liquid–liquid extraction [10], nowadays the generators, based on a solid matrix (inorganic or organic) providing "ionic" $^{68}Ga^{3+}$ eluates. The first commercially available generator of this type was developed by Cyclotron Ltd., Obninsk, Russian Federation [25] eluting gallium-68 with 0.1 M HCl with initial elution yields of ~80% and ^{68}Ge breakthrough of 0.001% [26]. Since the introduction of this generator in 1996 [26], a lot has happened on the market. Today several manufacturers produce ^{68}Ge/^{68}Ga-generators, including ones with GMP grade (e.g. Isotopen Technologien Garching (ITG)) or with approval (e.g. GalliPharm® Eckert & Ziegler in the EU with marketing authorization, in the USA with type II drug master file (DMF) on file with FDA).

Even though these generators represent considerable improvements in ^{68}Ga-production, there are still some obstacles to direct radiolabeling with gallium-68. Beside the low radioactive and high [H^+] concentration and ^{68}Ge breakthrough, especially the presence of other trivalent metal ions is an inconvenience. As 1 GBq gallium-68 is equal to 9.73 pmol (9.73×10^{-12} mol), these metallic impurities, even present at low levels (<ppm), can be a serious problem as they can compete with gallium-68 for the chelating function of the precursor. In addition to the IAEA recommendations on externally introduced metallic contaminations [21], several procedures are available to reduce those metallic impurities, either intrinsic or externally introduced. These post-elution purification methods, so called post-processing's, aim to improve the radioactive and [H^+] concentration and the radionuclidic as well as chemical purity of the ^{68}Ga-eluate. Beside fractionation of the eluate [11], anion-exchange (AEX) [13], cation-exchange (CEX) [27–29] and a combination thereof [30, 31] found to be suitable but only for fractionation but also are commercially used for cation-exchange.

6.2 Work in progress: cyclotron

Although ^{68}Ge/^{68}Ga-generators represent a convenient possibility for persistent patient care with ^{68}Ga-radiopharmaceuticals, their ^{68}Ga-activity available for

radiolabeling underlies several restrictions resulting from generator design and physics. In conjunction with the sharp increase in demand in recent years, alternative production routes, preferably realizable with existing medical cyclotrons, moved into the focus.

Small to medium energy medical cyclotrons are suitable for ^{68}Ga-production via the ^{68}Zn(p,n)^{68}Ga reaction using either a solid or a liquid target. Among the possible nuclear reactions [32, 33], it is the most reasonable leading to large production yields. For optimal results, the starting material zinc-68 as well as the proton energy needs to be selected with care to reduce co-production of long-living radioisotopes of gallium. Nevertheless, co-production of gallium-66 and gallium-67 is unavoidable due to the starting material and the excitation function of the ^{68}Zn(p,2n)^{67}Ga reaction [32, 33]. This has to be taken into account when producing gallium-68 via cyclotron for radiopharmaceutical application as both radioisotopes cannot be separated from the desired gallium-68.

For production of gallium-68 via cyclotron, either a solid or a liquid target can be used. For both target types, a lot of options exist leading to a several considerations to be made. Solid targets, for example, can be pressed, electroplated, foil or fused, all types having their advantages and disadvantages which are not mentioned here. In a first instance, the choice of target will mostly be done due to the actual conditions of the site. An existing production site for ^{18}F-compounds which want to implement gallium-68 would probably choose the liquid target route, as the preconditions for a solid target (target holder, cooling, target transfer and target processing) are expensive and likely not available. Compared with that, the liquid target is a quick and inexpensive option to obtain gallium-68 when a generator is not reasonable. A detailed overview about all possible alternatives and their advantages/disadvantages is given by the IAEA [21].

After irradiation, the gallium-68 needs to be purified from target material either if a solid or liquid target was used. The quantity of zinc necessary for the target need to be removed as it and all other metal impurities may perturb the radiolabeling reaction of gallium-68. Intense research on this topic lead to several purification methods based on solvent extraction [34, 35], precipitation [36] and solid phase separation [37–44] and suitable for automation.

Solid-phase extraction using a cation exchange resin or hydroxamate resin is most appropriate for an effective separation of gallium-68 from unwanted metals and can be easily combined with a second resin. This second purification step allows an additional reduction of [H^+] concentration to facilitate further processing of the final product [21]:

- Local conditions (expertise and equipment)
- Separation time (should be as short as possible)
- Acids (concentration and volume)
- Availability of materials
- Robustness of technique
- Ease of automation
- Possibility to recycle zinc-68 from target solution

7. Radiolabeling: complexation chemistry in clinical settings

7.1 Manual

The manual radiolabeling approach is a leftover from times, where gallium-68 was mainly used for research purpose, with lower ^{68}Ga-activities and not in a clinical setting for patient care. It is widely used in research and development of new tracers [11–13, 29, 30, 45–51]. Its main advantage is full control over the complete process (pH, time and temperature) and the possibility to easily access radiolabeling kinetics.

Due to its general setup, this method is not suitable and indented for clinical use. Nevertheless, before the introduction of module systems or the cold kits, it was a long time, the only available method.

In general (**Figure 4**), the first step is the preparation of the reaction mixture by mixing [^{68}Ga]$GaCl_3$ with a suitable buffer in the required pH range and the radiolabeling precursor. Here, the purified cyclotron-produced, generator eluate or post-processed gallium-68 can be used.

Then, the reaction vial is incubated to form the ^{68}Ga-complex. Reaction period and reaction temperature are selected in accordance to the kinetics of the complex formation of gallium with the used chelator.

After the reaction, the reaction mixture can be purified using, for example, solid phase extraction from, for example, free gallium-68 and residual germanium-68 impurities.

In the final step, the ^{68}Ga-radiopharmaceutical is sterile filtrated and formulated in the product vial (**Table 2**).

7.2 Module

With the growing interest for gallium-68 not only for research but also for clinical routine and patient care the need for pharmacopeia compliant preparation of ^{68}Ga-radiopharmaceuticals. This led to promotion of the automation of the traditional manual synthesis from which numerous semi- and fully automated devices have emerged. Today, those systems are designed with respect to Good

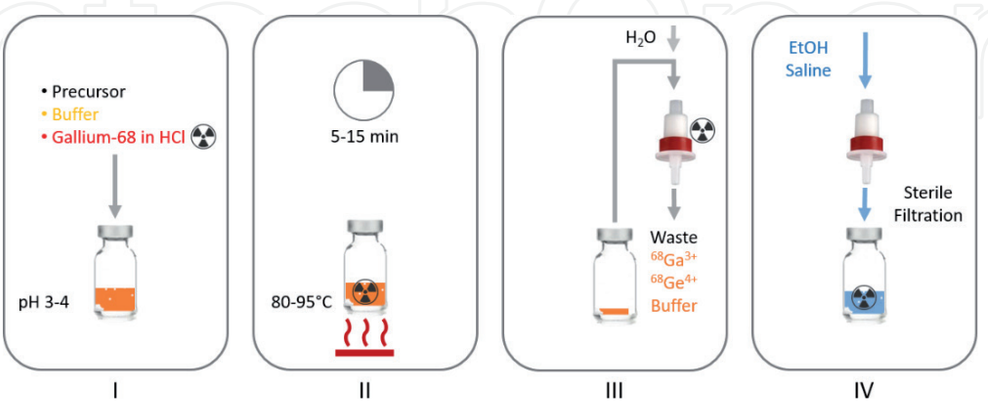

Figure 4.
Schematic description of the ^{68}Ga-radiolabeling procedure (I) preparation of the reaction mixture by adding gallium-68 eluted from a generator or after post-processing to a mixture of a suitable buffer and precursor, (II) incubation of the reaction mixture for a certain time. If elevated temperatures are needed or not depends on the chelator, (III) purification step using solid phase extraction (SPE). For example, the ^{68}Ga-radiopharmaceutical is trapped on a SPE C18-cartridge where it is washed with water to remove free gallium-68, germanium-68 and buffer, (IV) the purified product is finally eluted with diluted ethanol solution and formulated after sterile filtration in the product vial.

Chelator	Radiolabeling conditions
DOTA	37–90°C, 10–30 min, pH 4.0–5.5 [52, 53]
HBED	25°C, 10–20 min, pH 4.0–4.5 [54]

Table 2.
Radiolabeling conditions for gallium-68 for DOTA and HBED.

Manufacturing Practice (GMP) Guidelines provided, for example, by the FDA, EU/EMA, ICH, WHO or others [55]. They use software and methods designed to minimize user interventions and utilize single-use consumables produced under GMP standard.

While the module production requires a fully equipped laboratory and quality control, it reduces radiation exposure of the operator the production process in terms of higher reliability and reduced variability [56–58].

Accordingly, the amount of contaminated waste materials is higher due to the procedure as well the complete quality control. Nevertheless, these systems are suitable for a variety of tracers and in most cases for more radionuclides not only for gallium-68 (e.g. Scintomics GRP series; Eckert & Ziegler Modular-Lab PharmTracer; Trasis AllInOne).

7.3 Kits

Recently, cold kits for radiolabeling entered the scene enable production of ^{68}Ga-radiopharmaceuticals as easy as that of ^{99m}Tc-radiopharmaceuticals. This method allows the reconstitution of the pre-formulated cold kit with no previous post-processing of the eluate or subsequent purification of the final product. They are available in GMP quality and leaves only minimum quality control tests to the final user responsibility to verify the reconstitution procedure.

For example, the European Pharmacopeia (Ph. Eur.) states the marketing authorization (MA) holder of a licensed kit is responsible to ensure compliance of the kit with the requirements of its MA, while the final user carries the responsibility for the quality of the preparation and the handling. If the given instructions are not strictly followed or if one or more components used for the reconstitution do not have MA, it is the responsibility of the final user to demonstrate that the quality of the final preparation is suitable for the intended, use [26].

Therefore, preparation as well as quality control requires at least the equipment according to the instructions provided by the manufacturer. In addition, minimum contaminated waste materials remain. It has to be noted, according to the Ph. Eur. that applies only for licensed kits in combination with the generator mentioned in the instructions from the manufacturer. In contrast, unlicensed kits or a licensed kit used with an unlicensed generator or cyclotron produced gallium-68 also require full quality control according to the monograph. Additionally, local authorities may require more detailed quality control even for licensed kits.

Indeed, these cold kits contain relatively high amounts of precursor and additional filler materials. They still require manual handling and are only commercially available as single-dose kits for radiolabeling PSMA-11 (e.g. illumet™) and DOTA-TOC (e.g. NETSPOT®). In addition, the use of unpurified generator eluates requires very strict specifications for the generators in terms of ^{68}Ge-breakthrough to ensure the quality of the final product. Nevertheless, there is a possibility for small sites to offer ^{68}Ga-radiopharmaceuticals to their patients without great expense.

8. Quality control: pharmaceutical needs and radioactive specialties

Quality defects of pharmaceutical can lead to serious consequences when they are applied. Consequently, the regulatory framework for production and quality control is very strict. In general, one main requirement in the production of pharmaceuticals is a comprehensive, integrated system of quality assurance. Its purpose is the monitoring and documentation of all processes as well as their functionality with respect to the rules of GMP.

Because radiopharmaceuticals are pharmaceutical preparations containing minimum one radionuclide for diagnostic or therapeutic purpose, in principle the same rules apply. Their quality control is intended to ensure that the quality meets the predefined specifications for the radiopharmaceutical. These specifications take into account the radionuclide, the precursor, the preparation process, the formulation and the intended administration route. Due to the nature of the contained radionuclides, not all necessary quality control tests can be performed before release for administration and require retrospective examination. In the available monographs, it is indicated if a test need not to be completed before release of the batch.

In the case of gallium-68, the short half-live and the limited available activities lead to further challenges. Here are sophisticated logistics for preparation and quality control essential.

In general, quality control of ^{68}Ga-radiopharmaceuticals should include the following tests and information [59–61]:

1. *Characters/appearance*. Should discover any visible container defects. The quality of the final product in terms of absence of particular matter [62] and/or turbidity should be ensured as well as its correct appearance. Typically performed by visual inspection.

2. *pH determination*. Should ensure that the pH of the final product is in the necessary range for its purpose. For the final injectable formulation of a radiopharmaceutical, the pH should be closed to the physiologic value of 7.4. With regard to the relatively low volume of radiopharmaceuticals and depending on the injected volume and rate, a wider range (3.5–8.5) is applicable. Contrary to this, the pH of the radionuclide precursor gallium-68 should not exceed 2 to prevent the formation of unwanted ^{68}Ga-colloids.

3. *Radionuclidic identification*. Identification of a radionuclide is generally conducted by determination of its half-life and/the nature and energy of its radiation emitted. For positron emitters like gallium-68 instead of energy and nature of the radiation, the identification is based on a γ-spectrum additional to their half-life determination (e.g. with dose calibrator).

4. *Radiochemical identification.* Identification of the desired radiochemical species via HPLC and/or TLC exploiting different chemical behavior of the different radiochemical species.

5. *Radionuclidic purity*. Due to the contribution or formation of other radionuclides during the production of gallium-68, their amount present in the final radiopharmaceutical must be determined. Depending on the production route of gallium-68, different limits for radionuclidic impurities may apply. The test for those long-living radionuclides need to be performed after complete decay of the sample using γ-spectrometry, representing a test performed after release of the batch.

6. *Radiochemical purity*. Should discover all chemical forms containing the radionuclide and determine their percentage of the total radioactivity of the product. These radiochemical impurities arise from the synthesis method, radiolysis or the radionuclide production and can lower the quality of the final diagnostic examination. Principally be determined by any suitable analytical method but with respect to the short half-life and radiation TLC and HPLC are normally used for quality control of ^{68}Ga-radiopharmaceuticals.

7. *Chemical purity*. The chemical purity refers to the amount of the specified chemical form of a preparation if radioactivity is present or not [61]. Purity assessment is of special importance when diagnostic or therapeutic properties are directly linked to chemistry [63]. Therefore, particular attention is necessary for pharmacologically active impurities as they can affect the diagnostic value of the examination. The chemical purity of ^{68}Ga-radiopharmaceuticals is normally ascertained with TLC and/or HPLC.

8. *Residual solvents*. Ph. Eur. as well as US pharmacopeia defines residual solvents as organic volatile chemicals used in the manufacture of drug substances/active substances, excipients or in the preparation of medicinal products (Eur. Ph. 5.4.; USP 467). As they represent a risk of health, they should be determined. Determination can be performed using gas chromatography (GC)

It has to be noted that the texts about residual solvents not cover solvents added by purpose or solvates. For those other limits and regulations may apply.

9. *Microbiological contamination*. Parenteral administered radiopharmaceuticals need to be compliant in terms of bacterial endotoxins or pyrogens as well as sterility

Bacterial endotoxins are known to cause a wide spectrum of nonspecific pathophysiological reactions (fever, changes in white blood cell counts, hypotension, disseminated intravascular coagulation, shock and death) leading to death when injected in most mammals [64]. Thanks to the development of more and more efficient systems today tests (LAL-test) for bacterial endotoxins (BET) can be completed before release of the batch of the ^{68}Ga-radiopharmaceuticals.

In contrary, the test for sterility of ^{68}Ga-radiopharmaceuticals via direct inoculation is necessarily retrospective nevertheless indispensable. Additionally, to the direct inoculation test the integrity of the sterile filter used for sterile filtration of the final product is performed. Due to the need for sterilization to obtain a sterile parenteral solution and the not applicability of autoclaving for short-living radiopharmaceuticals membrane filtration is normally the method of choice. The tests for the filter integrity (e.g. bubble point, diffusion rate, pressure hold) have the advantage that they can be completed before batch release.

10. *Radioactivity content/concentration*. Defines the activity, measured with a dose calibrator, within the volume of the final preparation.

11. *Specific radioactivity*. The specific radioactivity (activity of the radionuclide per unit mass either of the element or the desired chemical form) is calculated using the concentrations of radioactivity and the chemical form. Referring to the consensus nomenclature rules for radiopharmaceutical chemistry [65], the specific activity is expressed as measured activity per gram of compound (e.g. MBq/μg), while it is called molar activity when expressing the measured

activity per mole of compound (MBq/nmol) [65]. As gallium-68 requires a complex ligand which is normally not fully removed during the final product purification, the measured specific or molar activity is lower than actual. Then the correct terms are apparent specific or molar activity [65].

The specific or molar activity is always given with reference date and time.

8.1 Generator obtained gallium-68

For gallium-68 obtained from a ^{68}Ge/^{68}Ga-generator, the Ph. Eur. contains a distinct monograph (#2464). This monograph specifies the quality characteristics of ^{68}Ga chloride solutions for radiolabeling independently if obtained directly from a generator or after post-processing the generator eluate. If a further purification of the generator eluate is performed, this has to be stated on the label.

Use of generator-produced gallium-68 in the USA is regulated under 10 CFR 35. 1000 and 10 CFR 30.33 [66] (**Table 3**).

For incoming starting materials, the GMP guidelines prescribe certain handling procedures to ensure their quality and suitability. For material acceptance of an incoming new ^{68}Ge/^{68}Ga-generator, minimum controls are needed. This include the conformation of the radionuclide identity, ^{68}Ge-breakthrough and of activity stated in the Certificate of Analysis (CoA) all verified by activity measurement if possible [60]. Establishment of additional acceptance criteria may be required.

Nevertheless, the ^{68}Ga-eluate used for radiolabeling should meet those specifications (**Table 4**), their verification is in clinical routine not possible for every production. This results from the different production routes of ^{68}Ga-radiopharmaceuticals, which do not intend or allow an intervention for sampling of the eluate. Thus, the quality control of the starting material gallium-68 or of the final radiopharmaceuticals is allowed. This should include at least tests for ^{68}Ge-breakthrough, radionuclidic purity, radiochemical purity and chemical purity.

8.2 Cyclotron produced gallium-68

When produced via accelerator, the presence of the radioisotopes gallium-66 and gallium-67 is difficult to avoid due to zinc-66 and zinc-67 contaminating the

WHAT?	HOW?	LIMITS
Appearance	Visual inspection	Clear, colorless solution
pH	pH indicator strips	<2
Radionuclide identity	Half-life determination	62–74 min
	γ-spectrometry	511, (1022), 1077, (18,839 keV
Radionuclidic purity	γ-spectrometry	<0.1% long living impurities
		<0.001% germanium-68
Radiochemical purity	TLC	>95% ^{68}Ga(III)
Chemical purity	ICP-AES/ICP-MS	<10 μg/GBq Fe
		<10 μg/GBq Zn
Bacterial Endotoxins	LAL test	≤175 EU/total volume

Table 3.
Quality control specifications for diluted hydrochloric solutions of generator produced gallium-68 as defined by the Ph. Eur. (monograph #2464) [59].

Manufacturer	Type	Maximum nominal activity
Eckert & Ziegler (Germany)	GalliaPharm®	2.4 GBq
	IGG100	2.4 GBq
Obninsk Cyclotron Ltd. (Russia)		3.7 GBq
IRE Elit (Belgium)	Galio Eo®	1.85 GBq
	Galli Ad®	1.85 GBq
ITG (Germany)		2 GBq
iThemba Labs (South Africa)		1.85 GBq
Pars Isotopes (Iran)	Pars-GalluGEN	2.59 GBq

Table 4.
In all conscience a list of ^{68}Ge/^{68}Ga-generators available.

target material. In return, germanium-68 is absent. Therefore, quality control and specifications for radionuclidic impurities are different to generator-produced gallium-68.

For gallium-68 obtained from a cyclotron, a new monograph (#3109) is already submitted for adoption to the Ph. Eur. [67]. This monograph specifies the quality characteristics of ^{68}Ga-chloride solutions for radiolabeling obtained by irradiation of enriched zinc-68 in an accelerator with subsequent isolation of gallium-68 in acidic solution (**Table 5**).

Similar to generator-produced gallium-68, quality control can be performed of the starting material obtained via cyclotron or on the final radiopharmaceutical. If quality control of the final radiopharmaceuticals performed, it should include at least tests for ^{68}Ge-breakthrough, radionuclidic purity, radiochemical purity and chemical purity.

8.3 ^{68}Ga-Radiopharmaceuticals

As an example for the specifications and limitations for a ^{68}Ga-radiopharmaceutical quality control as requested by the monograph #2464 of the

WHAT?	HOW?	LIMITS
Appearance	Visual inspection	Clear, colorless solution
pH	pH indicator strips	<2
Radionuclide identity	Half-life determination	62–74 min
	γ-spectrometry	511, (1022), 1077, (18,839 keV
Radionuclidic purity	γ-spectrometry	<0.1% long living impurities
		<2% gallium-66 & gallium-67
Radiochemical purity	TLC	>95% ^{68}Ga(III)
Chemical purity	ICP-AES/ICP-MS	<10 μg/GBq Fe
		<10 μg/GBq Zn
Bacterial Endotoxins	LAL test	≤175 EU/total volume

Table 5.
Quality control specifications for diluted hydrochloric solutions of accelerator-produced gallium-68 as defined by a draft of a monograph for the Ph. Eur. Submitted for adoption (#3109) [67].

WHAT?	HOW?	LIMITS
Appearance	Visual inspection	Clear, colorless solution
pH	pH indicator strips	< 2
Radionuclide identity	Half-life determination	62 to 74 min
	γ-spectrometry	511, (1022), 1077, (18,839 keV
Radionuclidic purity	γ-spectrometry	<0.1% long living impurities
		<0.001% germanium-68
Radiochemical purity	TLC	>91%
	TLC	<3% [^{68}Ga]Ga in colloidal form
	HPLC	<2% [^{68}Ga]Ga^{3+}
Chemical purity	ICP-AES/ICP-MS	<10 μg/GBq Fe
		<10 μg/GBq Zn
	HPLC	<50 μg/V DOTA-TOC and metal complexes of DOTA-TOC
	TLC	<200 μg/V HEPES
	GC	<10% V/V and <2.5 g per administration
Bacterial endotoxins	LAL test	≤175 EU/total volume
Sterility	Direct inoculation	sterile

Table 6.
Quality control specifications [^{68}Ga]Ga-DOTA-TOC as given by the Ph. Eur. For generator-produced gallium-68 (monograph #2464) [59].

Ph. Eur. [^{68}Ga]Ga-DOTA-TOC is provided [59]. It has to be noted, that monograph #2464 is currently under revision which can lead to different limits in feature (**Table 6**).

9. Regulatory aspects: the most important at the end

The quality control for a certain ^{68}Ga-radiopharmaceutical depends on the production route of gallium-68, the synthesis route of the radiopharmaceutical as well as of the relevant legislation.

As descripted in Section 7.2, the respective production route leads to different radionuclidic impurities (germanium-68 vs. gallium-66 & gallium-67) that need to take into account for the final product specifications. However, this is not yet implemented in the pharmacopeias but is in part already in progress. For example, the monograph for [^{68}Ga]Ga-DOTA-TOC (#2464) of the Ph. Eur. is currently in revision to take into account the cyclotron production of gallium-68 [68].

In general, the quality of the final radiopharmaceutical needs to fulfill all specifications given by the relevant legislation or pharmacopeia independent from the synthesis route. Nevertheless, it may be possible to dispense individual tests given, for example, for licensed kit preparations. For example, the Ph. Eur. states in its general notices "An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and data derived, for example, from

validation studies of the manufacturing process" [59]. Further details can be found in the general chapter on extemporaneous preparation of radiopharmaceuticals (5.19) and the general monograph radiopharmaceutical preparations (#0125) [59].

Nevertheless, the competent authorities may request further quality control testing. Therefore, it is strongly recommended, especially in case of doubt, to consult the competent authorities.

The implementation of a new radiopharmaceutical into the certain pharmacopeias is a protracted process. Therefore, several commonly used ^{68}Ga-radiopharmaceuticals are not yet represented with own monographs in the pharmacopeias (e.g. [^{68}Ga]Ga-PSMA-11). Nevertheless, such radiopharmaceuticals can be produced with consideration of the general notices, texts, monographs and along the lines of, for example, the monograph for [^{68}Ga]Ga-DOTA-TOC. Again, in case of doubt, the competent authorities should be consulted.

10. Conclusion

Gallium-68 is a well-researched radionuclide with growing importance for clinical practice triggered by the development of new tracers expanding its application and the increasing demand for theranostic patient care.

Its availability via radionuclide generator in combination with comparably easy coordination chemistry enables a patient care even in places where the cyclotron-produced PET-radionuclides are unavailable and, in the case of NETs, enables patient care where no ^{18}F-alternative exists.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

AEX	anion-exchange
API	active pharmaceutical ingredient
BET	bacterial endotoxin test
CEX	cation-exchange
DMF	drug master file
EU	European Union
FDA	U.S. Food and Drug Administration
GC	gas chromatography
GMP	good manufacturing practice
HCl	hydrochloric acid
HPLC	high pressure liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
ITG	Isotopen Technologien Garching

LAL-test	limulus amebocyte lysate test
M	molarity (mol/liter)
MA	marketing authorization
mCRPC	metastatic castrate-resistant prostate cancer
NET	neuroendocrine tumor
PC	prostate cancer
PET	positron emission tomography
Ph. Eur.	European pharmacopeia
pmol	picomol (10^{-12} mol).
QC	quality control
PRRT	peptide receptor radionuclide therapy
SPE	solid phase extraction
$T_{1/2}$	half-life
TLC	thin layer chromatography
USA	United States of America
U.S.	United States
USP	United States Pharmacopeia

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References

[1] Werner RA, Bluemel C, Allen-Auerbach MS, Higuchi T, Herrmann K. ^{68}Gallium- and ^{90}Yttrium–/^{177}Lutetium: "Theranostic twins" for diagnosis and treatment of NETs. Annals of Nuclear Medicine. 2015;**29**(1):1-7

[2] Strosberg J, Wolin E, Chasen B, Kulke M, Bushnell D, Caplin M, et al. Health-related quality of life in patients with progressive Midgut neuroendocrine tumors treated with 177Lu-Dotatate in the phase III NETTER-1 trial. Journal of Clinical Oncology. 2018;**36**(25):2578-2584

[3] Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 trial of 177Lu-Dotatate for Midgut neuroendocrine tumors. The New England Journal of Medicine. 2017;**376**(2):125-135

[4] Paganelli G, Sansovini M, Ambrosetti A, Severi S, Monti M, Scarpi E, et al. 177 Lu-Dota-octreotate radionuclide therapy of advanced gastrointestinal neuroendocrine tumors: Results from a phase II study. European Journal of Nuclear Medicine and Molecular Imaging. 2014;**41**(10):1845-1851

[5] Mahajan S, O'Donoghue J, Weber W, Bodei L. Integrating early rapid post-peptide receptor radionuclide therapy quality assurance scan into the outpatient setting. Journal of Nuclear Medicine and Radiation Therapy. 2019;**10**(1):395

[6] Carlsen EA, Fazio N, Granberg D, Grozinsky-Glasberg S, Ahmadzadehfar H, Grana CM, et al. Peptide receptor radionuclide therapy in gastroenteropancreatic NEN G3: A multicenter cohort study. Endocrine-Related Cancer. 2019;**26**(2):227-239

[7] Capdevila J, Fazio N, Lopez C, Teule A, Valle JW, Tafuto S, et al. 1307OEfficacy of lenvatinib in patients with advanced pancreatic (panNETs) and gastrointestinal (giNETs) grade 1/2 (G1/G2) neuroendocrine tumors: Results of the international phase II TALENT trial (GETNE 1509). Annals of Oncology. 2018;**29**(suppl_8)

[8] Bodei L, Cremonesi M, Grana CM, Fazio N, Iodice S, Baio SM, et al. Peptide receptor radionuclide therapy with ^{177}Lu-DOTATATE: The IEO phase I-II study. European Journal of Nuclear Medicine and Molecular Imaging. 2011;**38**(12):2125-2135

[9] Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): The NORDIC NEC study. Annals of Oncology. 2013;**24**(1):152-160

[10] Gleason GI. A positron cow. The International Journal of Applied Radiation and Isotopes. 1960;**8**(2-3):90-94

[11] Breeman WAP, Jong M, Blois E, Bernard BF, Konijnenberg M, Krenning EP. Radiolabelling DOTA-peptides with ^{68}Ga. European Journal of Nuclear Medicine and Molecular Imaging. 2005;**32**(4):478-485

[12] Velikyan I, Beyer GJ, Langström B. Microwave-supported preparation of ^{68}Ga bioconjugates with high specific radioactivity. Bioconjugate Chemistry. 2004;**15**(3):554-560

[13] Meyer G-J, Mäcke H, Schuhmacher J, Knapp WH, Hofmann M. ^{68}Ga-labelled DOTA-derivatised peptide ligands. European Journal of Nuclear Medicine and Molecular Imaging. 2004;**31**(8):1097-1104

[14] Lenzo NP, Meyrick D, Turner JH. Review of Gallium-68 PSMA PET/CT

imaging in the management of prostate cancer. Diagnostics (Basel). 2018;**8**(1):16

[15] Raj N, Reidy-Lagunes D. The role of 68Ga-DOTATATE positron emission tomography/computed tomography in well-differentiated neuroendocrine tumors: A case-based approach illustrates potential benefits and challenges. Pancreas. 2018;**47**(1):1-5

[16] Jansen TJP, van Lith SAM, Boss M, Brom M, Joosten L, Béhé M, et al. Exendin-4 analogs in insulinoma theranostics. Journal of Labelled Compounds and Radiopharmaceuticals. 2019;**62**(10):656-672

[17] Zhang J, Mao F, Niu G, Peng L, Lang L, Li F, et al. 68Ga-BBN-RGD PET/CT for GRPR and integrin αvβ3 imaging in patients with breast cancer. Theranostics. 2018;**8**(4):1121-1130

[18] Zhang J, Li D, Lang L, Zhu Z, Wang L, Wu P, et al. 68Ga-NOTA-Aca-BBN(7-14) PET/CT in healthy volunteers and glioma patients. Journal of Nuclear Medicine. 2016;**57**(1):9-14

[19] Velikyan I, Schweighöfer P, Feldwisch J, Seemann J, Frejd FY, Lindman H, et al. Diagnostic HER2-binding radiopharmaceutical, 68GaGa-ABY-025, for routine clinical use in breast cancer patients. American Journal of Nuclear Medicine and Molecular Imaging. 2019;**9**(1):12-23

[20] Schoffelen R, Sharkey RM, Goldenberg DM, Franssen G, McBride WJ, Rossi EA, et al. Pretargeted immuno-positron emission tomography imaging of carcinoembryonic antigen-expressing tumors with a bispecific antibody and a 68Ga- and 18F-labeled hapten peptide in mice with human tumor xenografts. Molecular Cancer Therapeutics. 2010;**9**(4):1019-1027

[21] IAEA. Gallium-68 Cyclotron Production. Vienna: International Atomic Energy Agency; 2019

[22] Velikyan I. Positron emitting 68GaGa-based imaging agents: Chemistry and diversity. Medicinal Chemistry. 2011;**7**(5):345-379

[23] McCutchan EA. Nuclear data sheets for a = 68. Nuclear Data Sheets. 2012;**113**(6-7):1735-1870

[24] Available from: http://www.nndc.bnl.gov/chart

[25] Razbash AA, Sevastianov YG, Krasnov NN, Leonov AI, Pavlekin VE, editor. Germanium-68 row of products. In: Proceedings of the 5th International Conference on Isotopes, 5ICI; Brussels, Belgium; 2005

[26] Dash A, Chakravarty R. Radionuclide generators: The prospect of availing PET radiotracers to meet current clinical needs and future research demands. American Journal of Nuclear Medicine and Molecular Imaging. 2019;**9**(1):30-66

[27] Mueller D, Klette I, Baum RP, Gottschaldt M, Schultz MK, Breeman WAP. Simplified NaCl based ^{68}Ga concentration and labeling procedure for rapid synthesis of ^{68}Ga radiopharmaceuticals in high radiochemical purity. Bioconjugate Chemistry. 2012;**23**(8):1712-1717

[28] Eppard E, Wuttke M, Nicodemus PL, Rösch F. Ethanol-based post-processing of generator derived ^{68}Ga towards kit-type preparation of ^{68}Ga-radiopharmaceuticals. Journal of Nuclear Medicine. 2014;**55**:1023-1028

[29] Zhernosekov KP, Filosofov DV, Baum RP, Aschoff P, Bihl H, Razbash AA, et al. Processing of generator-produced ^{68}Ga for medical application. Journal of Nuclear Medicine. 2007;**48**(10):1741-1748

[30] Mueller D, Klette I, Baum RP. The combined cationic-anionic purification of the ^{68}Ge/^{68}Ga generator eluate

for the labelling of fragile peptides. World Journal of Nuclear Medicine. 2011;**10**(1):73-89

[31] Loktionova NS, Belozub AN, Filosofov DV, Zhernosekov KP, Wagner T, Türler A, et al. Improved column-based radiochemical processing of the generator produced ^{68}Ga. Applied Radiation and Isotopes. 2011;**69**(7):942-946

[32] Gilly LJ, Henriet GA, Alves MP, Capron PC. Absolute cross sections and excitation functions for (d, p) and (d, 2n) reactions on Mn55 , Cu63, Cu65 , Zn66 , and Zn68 between 3 and 11.6 MeV. Physics Review. 1963;**131**(4):1727-1731

[33] Szelecsényi F, Kovács Z, Nagatsu K, Fukumura K, Suzuki K, Mukai K. Investigation of direct production of 68 Ga with low energy multiparticle accelerator. Radiochimica Acta. 2012;**100**(1):5-11

[34] Ugur Ö, Kothari PJ, Finn RD, Zanzonico P, Ruan S, Guenther I, et al. Ga-66 labeled somatostatin analogue DOTA-DPhe 1 -Tyr 3 -octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. Nuclear Medicine and Biology. 2002;**29**(2):147-157

[35] Lewis MR, Reichert DE, Laforest R, Margenau WH, Shefer RE, Klinkowstein RE, et al. Production and purification of gallium-66 for preparation of tumor-targeting radiopharmaceuticals. Nuclear Medicine and Biology. 2002;**29**(6):701-706

[36] Sadeghi M, Mokhtari L. Rapid separation of 67,68Ga from 68Zn target using precipitation technique. Journal of Radioanalytical and Nuclear Chemistry. 2010;**284**(2):471-473

[37] Alves F, Alves VHP, Do Carmo SJC, Neves ACB, Silva M, Abrunhosa AJ. Production of copper-64 and gallium-68 with a medical cyclotron using liquid targets. Modern Physics Letters A. 2017;**32**(17):1740013

[38] Lin M, Waligorski GJ, Lepera CG. Production of curie quantities of 68Ga with a medical cyclotron via the 68Zn(p,n)68Ga reaction. Applied Radiation and Isotopes. 2018;**133**:1-3

[39] Nair M. Cyclotron production and automated new 2-column processing of [68Ga]$GaCl_3$. European Journal of Nuclear Medicine and Molecular Imaging. 2017;**44**(Suppl 2):119-956

[40] Oehlke E, Hoehr C, Hou X, Hanemaayer V, Zeisler S, Adam MJ, et al. Production of Y-86 and other radiometals for research purposes using a solution target system. Nuclear Medicine and Biology. 2015;**42**(11):842-849

[41] Pandey MK, Bansal A, Engelbrecht HP, Byrne JF, Packard AB, DeGrado TR. Improved production and processing of ^{89}Zr using a solution target. Nuclear Medicine and Biology. 2016;**43**(1):97-100

[42] Pandey MK, Byrne JF, Jiang H, Packard AB, DeGrado TR. Cyclotron production of (68)Ga via the (68) Zn(p,n)(68)Ga reaction in aqueous solution. American Journal of Nuclear Medicine and Molecular Imaging. 2014;**4**(4):303-310

[43] Pandey MK, Byrne JF, Schlasner KN, Schmit NR, DeGrado TR. Cyclotron production of ^{68}Ga in a liquid target: Effects of solution composition and irradiation parameters. Nuclear Medicine and Biology. 2019;(**74-75**):49-55

[44] Engle JW, Lopez-Rodriguez V, Gaspar-Carcamo RE, Valdovinos HF, Valle-Gonzalez M, Trejo-Ballado F, et al. Very high specific activity ^{66}/^{68}Ga from zinc targets for PET. Applied Radiation and Isotopes. 2012;**70**(8):1792-1796

[45] Breeman WAP, de Blois E, Sze Chan H, Konijnenberg M, Kwekkeboom DJ, Krenning EP. ^{68}Ga-labeled DOTA-peptides and ^{68}Ga-labeled radiopharmaceuticals for positron emission tomography: Current status of research, clinical applications, and future perspectives. Seminars in Nuclear Medicine. 2011;**41**(4):314-321

[46] Eder M, Wängler B, Knackmuss S, LeGall F, Little M, Haberkorn U, et al. Tetrafluorophenolate of HBED-CC: A versatile conjugation agent for ^{68}Ga-labeled small recombinant antibodies. European Journal of Nuclear Medicine and Molecular Imaging. 2008;**35**(10):1878-1886

[47] Mathias CJ, Green MA. A convenient route to [68Ga]Ga-MAA for use as a particulate PET perfusion tracer. Applied Radiation and Isotopes. 2008;**66**(12):1910-1912

[48] Riss PJ, Kroll C, Nagel V, Rösch F. NODAPA-OH and NODAPA-(NCS)n: Synthesis, 68Ga-radiolabelling and in vitro characterisation of novel versatile bifunctional chelators for molecular imaging. Bioorganic and Medicinal Chemistry Letters. 2008;**18**(20):5364-5367

[49] Rösch F, Riss PJ. The renaissance of the ^{68}Ge/^{68}Ga radionuclide generator initiates new developments in ^{68}Ga radiopharmaceutical chemistry. Current Topics in Medicinal Chemistry. 2010;**10**(16):1633-1668

[50] Šimeček J, Hermann P, Wester H-J, Notni J. How is (68)Ga labeling of macrocyclic chelators influenced by metal ion contaminants in (68)Ge/(68)Ga generator eluates? ChemMedChem. 2013;**8**(1):95-103

[51] Velikyan I, Lendvai G, Välilä M, Roivainen A, Yngve U, Bergström M, et al. Microwave accelerated ^{68}Ga-labelling of oligonucleotides. Journal of Labelled Compounds and Radiopharmaceuticals. 2004;**47**(1):79-89

[52] Sun Y, Anderson CJ, Pajeau TS, Reichert DE, Hancock RD, Motekaitis RJ, et al. Indium (III) and gallium (III) complexes of bis(aminoethanethiol) ligands with different denticities: Stabilities, molecular modeling, and in vivo behavior. Journal of Medicinal Chemistry. 1996;**39**(2):458-470

[53] Wadas TJ, Wong EH, Weisman GR, Anderson CJ. Coordinating radiometals of copper, gallium, indium, yttrium, and zirconium for PET and SPECT imaging of disease. Chemical Reviews. 2010;**110**(5):2858-2902

[54] Taliaferro CH, Martell AE. New multidentate ligands. Xxvi. N,N' -BIS(2-hydroxybenzyl)ethylenediamine- N,N' -bis(methylenephosphonic acid monomethyl ester), and N,N' -bis(2-hydroxybenzyl) ethylenediamine- N,N' -bis (methylenephosphonic acid monoethyl ester): New chelating ligands for trivalent metal ions. Journal of Coordination Chemistry. 1984;**13**(3):249-264

[55] https://www.gmp-compliance.org/guidelines/gmp-guidelines

[56] Martin R, Jüttler S, Müller M, Wester H-J. Cationic eluate pretreatment for automated synthesis of [^{68}Ga] CPCR4.2. Nuclear Medicine and Biology. 2014;**41**(1):84-89

[57] 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 Oceania Journal of Nuclear Medicine and Biology. 2014;**2**(2):75-86

[58] Iori M, Capponi PC, Rubagotti S, Esposizione LR, Seemann J, Pitzschler R, et al. Labelling of 90 Y- and 177 Lu-DOTA-bioconjugates for targeted radionuclide therapy: A comparison among manual,

semiautomated, and fully automated synthesis. Contrast Media and Molecular Imaging. 2017;(7):1-12

[59] European Pharmacopoeia, 10th Edition 2019. Subscription to Main Volume + Supplement 1 + Supplement 2. 1st ed. Stuttgart: Deutscher Apotheker Verlag; 2019

[60] IAEA. Quality Control in the Production of Radiopharmaceuticals. Vienna: International Atomic Energy Agency; 2018

[61] WHO Pharmacopoeia Library. Available from: http://apps.who.int/phint/en/p/docf/

[62] Langille SE. Particulate matter in injectable drug products. PDA Journal of Pharmaceutical Science and Technology. 2013;**67**(3):186-200

[63] Pauli GF, Chen S-N, Simmler C, Lankin DC, Gödecke T, Jaki BU, et al. Importance of purity evaluation and the potential of quantitative ^{1}H NMR as a purity assay. Journal of Medicinal Chemistry. 2014;**57**(22):9220-9231

[64] Todar K, Madison WI. Bacterial Endotoxin. Available from: http://textbookofbacteriology.net/endotoxin.html

[65] Coenen HH, Gee AD, Adam M, Antoni G, Cutler CS, Fujibayashi Y, et al. Consensus nomenclature rules for radiopharmaceutical chemistry - setting the record straight. Nuclear Medicine and Biology. 2017;**55**:v-xi

[66] U.S. Nuclear regulatory Commision. Germanium-68/Gallium-68 Pharmaceutical Grade Generators Licensing Guidance. 2019. Available from: www.nrc.gov/docs/ML1910/ML19106A367.pdf

[67] Council of Europe. Pharmeuropa Online 30.4. Available from: http://pharmeuropa.edqm.eu/home/

[68] EDQM. Knowledge Database. Available from: https://extranet.edqm.eu/4DLink1/4DCGI/Web_View/mono/2464