The $^{68}$Ge/$^{68}$Ga generator provides an excellent source of positron-emitting $^{68}$Ga. However, newly available “ionic” $^{68}$Ge/$^{68}$Ga radionuclide generators are not necessarily optimized for the synthesis of $^{68}$Ga-labeled radiopharmaceuticals. The eluates have rather large volumes, a high concentration of $\text{H}^+$ (pH of 1), a breakthrough of $^{68}$Ge, increasing with time or frequency of use, and impurities such as stable Zn(II) generated by the decay of $^{68}$Ge, Ti(IV) as a constituent of the column material, and Fe(III) as a general impurity. **Methods:** We have developed an efficient route for the processing of generator-derived $^{68}$Ga eluates, including the labeling and purification of biomolecules. Preconcentration and purification of the initial generator eluate are performed using a miniaturized column with organic cation-exchanger resin and hydrochloric acid/acetone eluent. The purified fraction was used for the labeling of nanomolar amounts of octreotide derivatives either in pure aqueous solution or in buffers. **Results:** Using the generator post-eluate processing system, $>97\%$ of the initially eluated $^{68}$Ga activity was obtained within 4 min as a 0.4-mL volume of a hydrochloric acid/acetone fraction. The initial amount of $^{68}$Ge(IV) was decreased by a factor of $10^5$, whereas initial amounts of Zn(II), Ti(IV), and Fe(III) were reduced by factors of $10^6$, $10^5$, and 10, respectively. The processed $^{68}$Ga fraction was directly transferred to solutions containing labeling precursors—for example, DOTA-oPhe$^1$-Tyr$^4$-octreotide (DOTATOC) (DOTA = 1,4,7,10-tetraazacyclododecane-$N,N^\prime,N^\prime\prime,N^\prime\prime\prime$-tetraacetic acid). Labeling yields of $>95\%$ were achieved within 10 min. Overall yields reached $70\%$ at 20 min after generator elution relative to the eluted $^{68}$Ga activity, not corrected for decay. Specific activities of $^{68}$Ga-DOTATOC were 50 MBq/nmol using a standard protocol, reaching 450 MBq/nmol under optimized conditions. **Conclusion:** Processing on a cation-exchanger in hydrochloric acid/acetone media represents an efficient strategy for the concentration and purification of generator-derived $^{68}$Ga(III) eluates. The developed scheme guarantees high yields and safe preparation of injectable $^{68}$Ga-labeled radiopharmaceuticals for routine application and is easy to automate. Thus, it is being successfully used in clinical environments and might contribute to a new direction for clinical PET, which could benefit significantly from the easy and safe availability of the radionuclide generator-derived metallic positron-emitter $^{68}$Ga.

**Key Words:** $^{68}$Ge/$^{68}$Ga radionuclide generator; post-processing of eluates; cation-exchange chromatography; molecular imaging; PET/CT


**DOI:** 10.2967/jnumed.107.040378

---

**An important characteristic of positron-emitting $^{68}$Ga is its cyclotron-independent availability via the $^{68}$Ge/$^{68}$Ga radionuclide generator system. The relatively long-lived $^{68}$Ge (half-life $[t_{1/2}] = 270.95$ d) produces a short-lived $^{68}$Ga ($t_{1/2} = 67.71$ min), which subsequently decays to stable $^{68}$Zn. $^{68}$Ga is an excellent positron emitter, with $89\%$ positron branching accompanied by low photon emission (1,077 keV, 3.22%) ($I_2$). $^{68}$Ge/$^{68}$Ga radionuclide generators have been the object of development and investigation for almost 50 y. For a recent review on this and other PET radionuclide generator systems see Rösch et al. (3).

Today, the most common commercially available $^{68}$Ge/$^{68}$Ga radionuclide generator is based on a TiO$_2$ solid phase (Cyclotron Co. Ltd.) (4). The generators are produced with $^{68}$Ge activities of up to 3.7 GBq. “Ionic” $^{68}$Ga$^{3+}$ is eluted in 0.1N HCl solution. The $^{68}$Ga yield is $>60\%$ in 5 mL of the eluate; the $^{68}$Ge breakthrough usually does not exceed $5\times10^{-3}\%$.

The $^{68}$Ge/$^{68}$Ga radionuclide generator systems known today are not necessarily optimally designed for direct application in a medical context. The eluate from the commercial generator still contains measurable activities of long-lived $^{68}$Ge. In addition, the rather large volume and the relatively high concentration of hydrochloric acid in many cases prevent the direct use for labeling reactions. Furthermore, labeling yields and specific activities might not reach maximum values due to the presence of metallic impurities. For example, significant amounts of Zn(II) are generated from the decay of $^{68}$Ga. For a “fresh” 1,110-MBq generator, the number of stable $^{68}$Zn atoms generated within 1 d after an...
elution amounts to 8.93×10^{13} \text{ atoms (i.e., 10 ng of Zn(II))}
compared with 4.69×10^{12} \text{ atoms of } 68\text{Ga for 800 MBq } 68\text{Ga eluted. In addition, Ti(IV) or other residuals resulting from the generator column material and Fe(III) are present in the eluate. All of this will prevent achieving sufficient } 68\text{Ga-labeling yields and specific activities if low amounts of the labeling precursor are mandatory. Thus, dedicated procedures to process the initial radionuclide generator eluate—ideally including labeling and purification of } 68\text{Ga radiopharmaceuticals—need to be developed. Several approaches for processing generator-derived } 68\text{Ga(III) were described recently (5–8).}

Anion-Exchange Chromatography. In these cases (5,6), the initial volume of 10 mL of the 0.1N HCl eluate is transferred to a vial containing 15 mL of 9.5N HCl to obtain a final hydrochloric acid concentration of 5.5 M. Under these conditions, 68\text{Ga can be adsorbed on a strong anion-exchanger as anionic chloro complexes of } 68\text{Ga(III). After a washing step with 1 mL of 5.5N HCl, the resin is flushed with a stream of nitrogen and then eluted with } H2O
in small volumes. This strategy separates 68\text{Ga but does not allow direct loading of } 68\text{Ga(III) on the anion-exchange resin from 0.1N HCl and it does not provide sufficient purification of } 68\text{Ga(III) from, for example, Zn(II) and Fe(III). The time needed to process the generator eluate, to synthesize and to purify, for example, } 68\text{Ga-labeled DOTA-conjugated peptides (DOTA = 1,4,7,10-tetraazacyclododecane-N,N',N''-tetraacetic acid), and the efficiency related to each of the individual steps define an overall practical yield of generator eluate processing before subsequent synthesis of >94% and of about 46% ± 5% for the } 68\text{Ga-labeled DOTA-conjugated octreotide, both referred to the initially eluted activity of } 68\text{Ga (9).}

Fractionation. Another approach to overcome problems such as eluate volume, acidic pH, and content of 68\text{Ge and chemical impurities is to fractionate the initial generator eluate (8). The concept uses the fact that the eluted } 68\text{Ga activity peaks within ~1–2 mL, representing about two thirds of the total activity. In the context of synthesizing } 68\text{Ga-labeled compounds, decay-corrected yields of } 68\text{Ga radiopharmaceuticals thus cannot exceed 60%–70%. Due to processing times and labeling efficacies, in practice, effective yields of labeled DOTA-conjugated peptides such as } 68\text{Ga-DOTATOC amount to about 50% (10). Contents of } 68\text{Ge and metallic impurities are lowered because of the lower eluate volume used but principally not chemically removed before the } 68\text{Ga-labeling steps.}

Strategy of the Present Approach. The aim of this work was to develop an efficient and simplified system for processing } 68\text{Ge/68\text{Ga generator–produced } 68\text{Ga eluates adequate for clinical requirements. It combines volume reduction with chemical and radiochemical purification. This leads to almost complete removal of metallic impurities and } 68\text{Ge breakthrough, thus providing the purified } 68\text{Ga in a form useful for direct labeling (acceptable pH, volume, purity). It allows for preparation of injectable } 68\text{Ga-labeled radiopharmaceuticals using a generator-associated, combined processing/labeling/purification module for easy routine use in a clinical environment.}

The key step consists in the direct transfer of the initial 0.1N HCl 68\text{Ga eluate to a cation-exchanger. Due to high distribution coefficients, generator-produced } 68\text{Ga can be quantitatively adsorbed on the resin directly from the generator eluate. Literature data indicate that, with the increase of the content of acetone in the HCl solutions of the same acid concentration, the affinity of } 68\text{Ga for the organic cation-exchange resin decreases (11,12). Consequently, systematic experiments have been performed in the present study to adapt this approach for processing generator-produced } 68\text{Ga. In addition, literature data do not necessarily provide separation conditions for tetravalent germanium and titanium, divalent zinc, and trivalent iron. Thus, it was another aim of this work to investigate the composition of hydrochloric acid/acetone solutions under which trivalent gallium can not only be concentrated but also be purified from Ge(IV), Ti(IV), Zn(II), and Fe(III).}

**MATERIALS AND METHODS**

**68\text{Ge/68\text{Ga Radionuclide Generator**}

Commercial generators based on a TiO2 phase adsorbing 68\text{Ge(IV) were obtained from Cyclotron Co. Ltd. In the present study, 740- and 1,110-MBq devices were used.}

**Radiometals Used for Ion-Exchange Distribution Measurements**

Ga(III): 110 MBq of 68\text{Ga in 7 mL of 0.1N HCl were obtained from a 1-y-old 740-MBq generator after >200 elutions. Ge(IV): The activity of 68\text{Ge in the } 68\text{Ga eluate used was about 170 kBq.}

Fe(III): 59\text{Fe was produced in a neutron-capture reaction on natural iron. One hundred ninety-eight milligrams of iron oxide Fe2O3 were irradiated for 50 d at the Hahn-Meitner-Institut (HMI) neutron source at } 1.6 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}; \text{ yielding } 440 \text{ MBq } 59\text{Fe. The iron oxide was dissolved in } HNO3 \text{ solution and, after evaporation, transferred into appropriate solutions.}

Zn(II): 69\text{Zn was produced with a specific activity of ~700 kBq/mg by irradiation of 380 } \mu\text{g of } 68\text{Zn (as Zn(NO3)2), >98% isotonically enriched } 69\text{Zn} \text{ for 6 h at the TRIGA II reactor Mainz at a neutron flux of } 4 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}. \text{ The target was subsequently dissolved in 0.1N HCl.}

The absolute activity of the radionuclides was analyzed by γ-spectrometry using a high-purity germanium (HPGe) detector.

**Chemicals and Equipment**

Analytic-reagent grade chemicals and Milli-Q water (18.2 MΩ-cm; Millipore) were used. Bio-Rad AG 50W-X8 cation-exchanger <400 mesh was used to prepare microchromatography columns (50 mg resin, ~2-mm inner diameter, ~5-mm length). For labeling reactions, 11-mL glass vials (Mallinckrodt) were used. A heating block with 1.5-cm lead thickness for radiation adsorption was built using 24-V Peletier Thermal Cycler heating elements. For processing of the labeling product, C-18 cartridges, Phenomenex Strata-X Tubes, 30 mg were used. Sterile filtration was done on 0.22-μm membrane filters (Millex GV).
For labeling of octreotide derivatives, DOTA-\(\text{d-Phe}^1\text{-Tyr}^3\)-conjugated octreotide (DOTATOC) was used.

**Experiments**

*Distribution of Metal Cations.* A microchromatography column was prepared using 50 mg of the resin. The distribution of \(^{68}\text{Ge(IV)}, \text{Fe(III)}, \text{Zn(II)}, \text{and Ti(IV)}\) on this cation-exchange column was investigated to quantitatively evaluate the possibility of purification of the generator produced \(^{68}\text{Ga}\) from contaminants.

The \(^{68}\text{Ga}\) eluted with \(5\)–\(10\) mL of \(0.1\text{N HCl}\) was transferred on line within 1–2 min on the chromatographic column. This represents the basic step to recover radioferrum from the generator eluate and to remove the main parts of the chemical and radiochemical impurities.

In a second step, the column was eluted with hydrochloric acid/acetone solutions of 80% acetone and HCl concentrations of 0.10N, 0.15N, or 0.20N. The volumes of the investigated mixtures ranged from 0.6 to 5.0 mL. The eluant remaining on the cation-exchanger was removed by passage of air. These 2 processes aimed to remove most of the remaining chemical and radiochemical impurities from the resin, whereas \(^{68}\text{Ga(III)}\) should quantitatively remain on the column.

Third, the column was filled with 150 \(\mu\text{L}\) of a 97.6% acetone/0.05N HCl solution. About 2 min standing appeared to be best for complete desorption of the \(^{68}\text{Ga(III)}\) from the resin into the liquid phase. An additional 250 \(\mu\text{L}\) of this mixture were applied, and the purified \(^{68}\text{Ga(III)}\) was obtained in 400 \(\mu\text{L}\) of this eluent overall. The column was reconditioned with 1 mL of 4N HCl and 1 mL of \(\text{H}_2\text{O}\).

Thus, 5 fractions were obtained and analyzed for their \(^{68}\text{Ga}\) and \(^{68}\text{Ge}\) contents (Table 1): (i) \(7\) mL \(0.1\text{N HCl}\); (ii) 80% acetone/0.10–0.20N HCl solutions; (iii) \(97.6%\) acetone/0.05N HCl solution; (iv) \(4\) N HCl; (v) \(\text{H}_2\text{O}\).

To analyze the behavior of the relevant impurities, the distributions of \(^{59}\text{Fe}\) in \(83\ \mu\text{g Fe(III)}\) and of \(^{69}\text{Zn}\) in \(130\ \mu\text{g Zn(II)}\) were determined using the same protocol. In addition, the behavior of stable Ti(IV) was investigated to estimate the distribution of Ti(IV) coeluted in the \(^{68}\text{Ga}\) fraction. Thus, 20 \(\mu\text{g Ti(IV)}\) in 5 mL \(0.1\text{N HCl}\) was processed the same way. The distribution of Ti(IV) in the different fractions was studied by an Elan 5000 ICP-MS (Perkin-Elmer).

*Labeling.* After preconcentration and purification of the initial generator eluates on the microchromatography column, \(^{68}\text{Ga(III)}\) was eluted with the \(400-\mu\text{L} \ 97.6\%\text{ acetone/0.05N HCl}\) solution (2 \(10^{-3}\) mol HCl). This fraction was used directly for the labeling of DOTATOC—that is, the processed activity was added to 4.5 mL pure \(\text{H}_2\text{O}\) in a standard glass reagent vial (11 mL; Mallinckrodt) containing 7–14 nmol of DOTATOC. No buffer solution was added.

Parallel experiments, however, were done with additional buffer solutions using 0.5–0.7 mL of 1 M N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) (HEPES), pH 4.0–4.1, in 2-mL reaction vessels (polypropylene brand equipped with a vent) containing 2–5 nmol of DOTATOC only.

All labeling reactions were performed at temperatures of \(\sim98^\circ\text{C}\). The kinetics of the complexation were recorded by taking aliquots of 1 mL at 1, 2, 5, and 10 min.

**TABLE 1**

Relative Distribution of Ga(III), Ge(IV), Zn(II), Ti(IV), and Fe(III) on Microchromatographic Column (50 mg of Bio-Rad AG 50W-X8 resin, 400 Mesh) in Hydrochloric Acid/Acetone Media

<table>
<thead>
<tr>
<th>Volume</th>
<th>Step</th>
<th>Eluent (concentration)</th>
<th>Ge(III)</th>
<th>Ge(IV)</th>
<th>Zn(II)</th>
<th>Ti(IV)</th>
<th>Fe(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>7 mL</td>
<td>Generator elution</td>
<td>0.10N HCl</td>
<td>0.19</td>
<td>98.14</td>
<td>0.61</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>5 mL</td>
<td>Purification</td>
<td>80% acetone/0.10N HCl</td>
<td>0.58</td>
<td>1.83</td>
<td>99.39</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>0.4 mL</td>
<td>(Gal) elution</td>
<td>97.6% acetone/0.05N HCl</td>
<td>98.50</td>
<td>2.10–2</td>
<td>5.10–2</td>
<td>5.10–2</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>4N HCl</td>
<td>0.53</td>
<td>6.10–3</td>
<td>&lt;10−3</td>
<td>90.92</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>(\text{H}_2\text{O})</td>
<td>0.20</td>
<td>5.10–3</td>
<td>&lt;10−3</td>
<td>1.01</td>
</tr>
<tr>
<td>Set 2</td>
<td>7 mL</td>
<td>Generator elution</td>
<td>0.10N HCl</td>
<td>0.16</td>
<td>97.08</td>
<td>0.77</td>
<td>7.30</td>
</tr>
<tr>
<td></td>
<td>0.6 mL</td>
<td>Purification</td>
<td>80% acetone/0.15N HCl</td>
<td>1.43</td>
<td>2.92</td>
<td>98.15</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>0.4 mL</td>
<td>(Gal) elution</td>
<td>97.6% acetone/0.05N HCl</td>
<td>97.82</td>
<td>3.10–2</td>
<td>1.08</td>
<td>7.10–2</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>4N HCl</td>
<td>0.41</td>
<td>5.10–3</td>
<td>&lt;10−3</td>
<td>72.15</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>(\text{H}_2\text{O})</td>
<td>0.18</td>
<td>3.10–3</td>
<td>&lt;10−3</td>
<td>19.80</td>
</tr>
<tr>
<td>Set 3</td>
<td>7 mL</td>
<td>Generator elution</td>
<td>0.10N HCl</td>
<td>0.11</td>
<td>97.68</td>
<td>0.43</td>
<td>7.18</td>
</tr>
<tr>
<td></td>
<td>5 mL</td>
<td>Purification</td>
<td>80% acetone/0.15N HCl</td>
<td>6.29</td>
<td>2.32</td>
<td>99.57</td>
<td>3.99</td>
</tr>
<tr>
<td></td>
<td>0.4 mL</td>
<td>(Gal) elution</td>
<td>97.6% acetone/0.05N HCl</td>
<td>92.73</td>
<td>5.10–3</td>
<td>&lt;10−3</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>4N HCl</td>
<td>0.77</td>
<td>2.10–3</td>
<td>&lt;10−3</td>
<td>86.54</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>(\text{H}_2\text{O})</td>
<td>0.10</td>
<td>2.10–3</td>
<td>&lt;10−3</td>
<td>2.18</td>
</tr>
<tr>
<td>Set 4</td>
<td>7 mL</td>
<td>Generator elution</td>
<td>0.10N HCl</td>
<td>0.29</td>
<td>98.08</td>
<td>0.92</td>
<td>6.33</td>
</tr>
<tr>
<td></td>
<td>0.6 mL</td>
<td>Purification</td>
<td>80% acetone/0.20N HCl</td>
<td>6.34</td>
<td>1.86</td>
<td>96.58</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>0.4 mL</td>
<td>(Gal) elution</td>
<td>97.6% acetone/0.05N HCl</td>
<td>92.96</td>
<td>5.10–2</td>
<td>3.70</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>4N HCl</td>
<td>0.28</td>
<td>6.10–3</td>
<td>&lt;10−3</td>
<td>87.37</td>
</tr>
<tr>
<td></td>
<td>1 mL</td>
<td>Washing</td>
<td>(\text{H}_2\text{O})</td>
<td>0.13</td>
<td>6.10–3</td>
<td>&lt;10−3</td>
<td>5.08</td>
</tr>
</tbody>
</table>

**68Ge/Ga Generator Post-processing • Zhernosekov et al.**
aluminum-backed silica gel 60 and 0.1 M Na₃citrate water solution as a mobile phase were selected. A pH of ~5 of the eluent was found to be optimum, providing Rₑ = 0.9 for free ⁶⁸Ga(III) while ⁶⁸Ga-DOTATOC remains at the origin. For HPLC, a Machery Nagel column (Nucleosil 5 C18-AB, 250 x 4 mm) and 20% AcCN, 80% trifluoroacetic acid/0.01% H₂O (flow rate, 1 mL/min under isocratic conditions) were used. Retention times were about 2 and 9 min for free ⁶⁸Ga(III) and ⁶⁸Ga-DOTATOC, respectively.

**Purification of ⁶⁸Ga-Labeled Peptide.** ⁶⁸Ga-Labeled DOTAOctreotide was purified from unreacted ⁶⁸Ga species by reversed-phase chromatography. The reaction mixture was passed through a small C18 cartridge (Phenomenex Strata-X tubes, 30 mg), providing quantitative recovery of the peptide on reverse phase. After washing the cartridge with 5 mL H₂O, the ⁶⁸Ga-labeled peptide was recovered with 200–400 µL of pure ethanol.

**Determination of Acetone Contents.** The acetone contents in the reaction mixture and in the final product were analyzed by gas chromatography (Hewlett Packard 6890 series gas chromatography system). According to the eluate processing and labeling protocols, 400 µL of a 97.6% acetone/0.05N HCl solution were added to 4.5 mL of preheated (~98°C) water in an open reaction vial (11 mL; Mallinkrodt). The acetone content in this solution was measured at different time points during incubation. In addition, the acetone concentration in the solutions after processing on the C18 cartridge was determined. The cartridge was washed with 5 mL of water and with 0.5 mL of ethanol. Finally, the ethanol fraction containing the labeled product was analyzed.

**RESULTS**

**Eluate Purification: Chemical and Radiochemical Purities**

Relative distributions of ⁶⁸Ga(III), ⁶⁶Ge(IV), Zn(II), Ti(IV), and Fe(III) on a microchromatography column (50 mg AG 50W-X8, 400 mesh) in hydrochloric acid/acetone media are summarized in Table 1.

<table>
<thead>
<tr>
<th>Element</th>
<th>Distribution</th>
<th>% of Initial Generator Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>⁶⁸Ga</td>
<td>99%</td>
<td>0.0%</td>
</tr>
<tr>
<td>⁶⁶Ge</td>
<td>0.1%</td>
<td>100%</td>
</tr>
<tr>
<td>Zn</td>
<td>0.1%</td>
<td>99%</td>
</tr>
<tr>
<td>Ti</td>
<td>0.1%</td>
<td>99%</td>
</tr>
<tr>
<td>Fe</td>
<td>0.1%</td>
<td>99%</td>
</tr>
</tbody>
</table>

**68Ga:** The microchromatographic column provides quantitative adsorption of >99% of ⁶⁸Ga from the initial generator eluate. An additional purification step with 80% acetone/HCl solutions causes some loss of ⁶⁸Ga activity. The part of ⁶⁸Ga, lost per milliliter volume of this eluent, increased with increasing acid concentration and was <7% in 0.6 mL (~12% ⁶⁸Ga/mL) with 0.20N HCl. The same activity was obtained in 5 mL with 0.15N HCl (~1.4% ⁶⁸Ga/mL) and only about 0.6% in 5 mL with 0.10N HCl (~0.1% ⁶⁸Ga/mL) (Table 1). Under those conditions (set 2), >99% of the ⁶⁸Ga initially remaining on the cation-exchanger could be eluted in 400 µL of the 97.6% acetone/0.05N HCl solution.

**68Ge:** ⁶⁸Ge(IV) passes through the column in the original 0.10N HCl solutions. The amount remaining in the free volume of the cation-exchanger column is further reduced by washing with the 80% acetone/HCl solutions. As a result, the final ⁶⁸Ga fraction contains <0.01% of ⁶⁸Ge relative to the initial eluate, independent of the HCl concentration of the 80% acetone solutions. Thus, by post-processing, an additional decontamination factor of ~10⁴ could be achieved for ⁶⁸Ge. Relative to an ~0.005% original ⁶⁸Ge generator breakthrough, for example, a very low overall content of ⁶⁸Ge in the processed ⁶⁸Ga fraction of ~5 x 10⁻⁷% is achieved. This represents a significant step toward a medical radionuclide generator approvable for direct clinical use.

Zn: Divalent zinc is quantitatively adsorbed (>99%) from the 0.10N HCl eluate along with trivalent gallium. However, Zn(II) is desorbed by the 80% acetone/HCl purification step, whereas ⁶⁸Ga(III) remains on the resin. The highest reduction of the zinc amount could be achieved applying 5 mL of an 80% acetone/0.15N HCl solution. The final content of Zn(II) in the processed ⁶⁸Ga(III) fraction of 400 µL of the 97.6% acetone/0.05N HCl solution is <10⁻³%.

Ti: Ti(IV) is almost completely adsorbed on the cation-exchange resin directly from the 0.1N HCl solution. Approximately 0.1% of Ti(IV) is eluted in 400 µL of the 97.6% acetone/0.05N HCl solution. The remaining Ti(IV) is eluted from the cation-exchange resin almost completely in 4N HCl, which allows the column to be cleaned.

Fe: The best separation factors are provided at an HCl concentration of 0.10N, but a large volume of the eluent must be applied because the distribution coefficient of iron is still high. Only about 54% of Fe(III) could be obtained in 5 mL of the 80% acetone/0.10N HCl solution (set 1), accompanied by ~0.6% of ⁶⁸Ga. At an HCl concentration of 0.20N (set 4), the mixture has evidently a higher elution capability for both cations. Here, 54% of iron and 6% of ⁶⁸Ga were eluted already in 0.6 mL. Application of an 80% acetone/0.15N HCl solution (set 2) seems to be an optimum, providing the best ratio of eluted amounts of iron relative to the loss of gallium. It was 38% of Fe(III) compared with 1.4% of Ga in 0.6 mL of the eluate. Five milliliters of the solution allowed washing down almost 90% of Fe(III) and about 6% of ⁶⁸Ga(III), resulting in the best decontamination factor for iron.

**Labeling**

The preconcentrated and purified ⁶⁸Ga(III) eluted from the resin with 400 µL of the 97.6% acetone/0.05N HCl mixture was used for labeling reactions. For labeling in pure water, up to 700 MBq ⁶⁸Ga were added to 4–4.5 mL (preheated) H₂O in open standard reagent vials containing 7–14 nmol of DOTA-Tyr. The amount of HCl contained in the final eluate solution (400 µL 97.6% acetone/0.05N HCl = 2.10⁻⁵ mol H⁺) provided an overall pH of 2.30 ± 0.05. At ~98°C and under these acidic conditions, radio labeling yield was ~95% within 10 min. Unstable radiochemical yields and increased adsorption on the glass surface were detected if <14 nmol of DOTA-Tyr was used. Incorporation also dropped with decreasing reaction temperature.

Specific activities of up to 50 MBq/nmol could be achieved. To increase the specific activity, additional experiments were performed in HEPES buffer systems using lower amounts of DOTA-Tyr. The processed ⁶⁸Ga eluate of 2 combined generators (2·10⁻⁵ mol HCl; up to 1.400 MBq ⁶⁸Ga) was added to 0.5–0.7 mL 1 molal HEPES solutions.
of pH 4.0–4.1 in 2-mL reaction vessels containing 2–4 nmol of DOTATOC, resulting in the mixture pH of 3.7–3.9. The radiochemical yield was up to 88% at ~9°C within 10 min, and the specific activity was up to 450 MBq/nmol.

**Post-processing and Syntheses Using a Prototype of a Module for Clinical Application**

The developed eluate processing and labeling protocol were used to design a prototype of a module system of generator post-processing (Fig. 1). A microchromatography column with about 50 mg of the cation-exchanger was prepared using two 3-way valves (I and II). The 68Ge/68Ga radionuclide generator was connected to the first valve (line 1). PEEK capillary tubing (line 4) was directed to the reagent vials in a heating block. The column could be eluted using a standard single-use syringe (position 3) and was connected to the waste vial (line 2). A small C18 cartridge (Phenomenex Strata-X tubes, 30 mg) was attached to the system (line 5) and was also operated using standard single-use syringes.

The processing of the generator eluate included several steps.

**Elution of Generator with 0.1N HCl.** As there is no need to limit the elution volume and, thereby, lose some of the 68Ga activity available at the radionuclide generator, 10 mL of 0.1N HCl were always injected into the generator. The eluate was passed directly through the microchromatography column (line 1) and transferred into the waste vial (line 2). This step provided recovery of >99% of 68Ga from the eluate on the cation-exchanger resin.

**Purification Step.** After switching the 3-way valve (I), the column was washed, using a single-use syringe (line 3) with 1 mL of the 80% acetone/0.15N HCl solution. The solution removed 68Ge(IV), which remained in the dead volume of the column, Zn(II), and Fe(III) and was collected in the waste vial (line 2). The remaining 0.80% acetone/0.15N HCl solution was removed from the cation-exchange resin by blowing air using a single-use syringe. The loss of 68Ga activity was <3%.

**Elution of Purified 68Ga Activity into Reaction Vial.** After switching the 3-way valve (II), the column was eluted using a single-use syringe filled with 400 μL 97.6% acetone/0.05N HCl solution into the reaction vial (line 4). The column was first filled with 150 μL of the solution and, after 2 min, 68Ga was washed off with the rest of the mixture. The column was freed of the remaining solution again using air from a single-use syringe. 68Ga was recovered from the resin with >99% yield—that is, <1% of the 68Ga adsorbed on the resin was lost.

After transfer of 68Ga into the labeling vial, the microchromatography column is reconditioned with 1 mL 4N HCl and 1 mL H2O (lines 2 and 3).

**Complexation.** Complexation is performed in unbuffered water as described earlier. The activity was eluted directly into the reaction vial (11 mL; Mallinckrodt) containing 14 nmol of DOTATOC in 4.5 mL of preheated (~98°C) water. Radiochemical yields of >95% could be achieved for 68Ga-DOTATOC syntheses within 10 min. The reaction mixture was passed through a small C18 cartridge (Phenomenex Strata-X tubes, 30 mg), using an empty syringe (line 5) providing quantitative absorption of the peptide on the cartridge. After washing the cartridge with 5 mL H2O (line 5), the 68Ga-labeled peptide was recovered from the cartridge with 200–400 μL of pure ethanol (line 6). Less than 0.5% of the activity in the ethanol eluate corresponded to free gallium. A radiochemical purity of >99.5% was obtained by processing the labeling mixture on a RP 18 cartridge. Thus, a radiochemically pure product, which did not depend on the initial radiolabeling yields, was guaranteed.

The ethanol eluate containing pure 68Ga-DOTATOC was dissolved in an appropriate volume of sterile 0.9% saline for medical application (line 6) and sterilized by filtration (line 7) through a 0.22-μm membrane filter. The developed scheme allowed the preparation of injectable 68Ga-labeled DOTA-peptides within 20 min, with overall yields up to 70% in reference to the initially eluted gallium activity, not corrected for decay (80% as corrected for decay). For a fresh 1,110-MBq generator, the batch activity of 68Ga-DOTATOC was 700 MBq before sterile filtration. The specific activity depended on the actual generator activity.

![FIGURE 1. Scheme (top) and post-processing of 68Ga-eluates and generator-associated syntheses of 68Ga-labeled compounds (e.g., labeling and product purification for DOTATOC as a proof-of-principle precursor).](image-url)
(as the peptide amount remains constant) and was up to 40 MBq/nmol for the unbuffered water labeling system compared with ~450 MBq/nmol for HEPES buffer systems with low peptide amounts.

**Routine Quality Control**

A radiochemical purity of >99.5% was guaranteed by processing the labeling solution on RP18 cartridges independent of the initial radiolabeling yield. However, routine quality control for the radiochemical purity is recommended in addition. It can be performed rapidly by TLC or HPLC.

**Content of Acetone**

The total amount of acetone within the 400 μL 97.6% acetone/0.05N HCl mixture transferred to the labeling vial is ~200 mg. Acetone evaporates while the labeling mixture is kept at ~95°C in the open vial. After 10 min, about 4.5 μg acetone remained in the reaction mixture. After purification of the 68Ga-labeled peptides on the RP18 cartridge, the overall acetone content in the final, injectable solution was <0.5 μg.

Those numbers are far below the intravenous toxicity of acetone in rats or mice (median lethal dose [LD50] > 1 g/kg (13,14)). Acetone is classified as a solution with a negligible intravenous toxicity of 5,500 mg/kg (rat) (14).

**DISCUSSION**

Radiolabeled DTPA- and DOTA-conjugated peptides of high affinity and selectivity for tumor membrane receptors applied to SPECT of various tumors clearly have indicated the great scientific and diagnostic potential of this particular molecular targeting. Recently, these applications have been expanded by the availability of new 68Ga-labeled analogs. The favorable nuclear decay parameters of 68Ga (t1/2 = 67.71 min, 89% β+ branching) allow the application of the most potent nuclear medical imaging technology—that is, PET—for the routine diagnosis of neuroendocrine and other tumors. The identification of solid tumors and disseminated soft-tissue and bone metastases has reached a completely new level of highly accurate tumor diagnosis, especially if PET/CT is used. Consequently, there is an increasing demand for commercially available 68Ge/68Ga radionuclide generators, ready for routine clinical application.

A commercial 68Ge/68Ga radionuclide generator system based on a TiO2 matrix is available from Cyclotron Co., Ltd. and provides high and stable elution yields. Because of the large volume of the eluate and its high acidity, its contents of chemical impurities such as Zn(II), as generated from the decay of 68Ga, Ti(IV) or other residuals representing the generator column material, and Fe(III), as well as its breakthrough representing long-lived 68Ge, the initial generator eluate might fail to provide sufficient 68Ga-labeling yields and specific activities if low amounts of the labeling precursor are mandatory. Moreover, it might limit its wide application for routine patient diagnosis not only because of the chemical aspects but also because of requirements related to legal considerations for radiopharmaceutical registration.

As demonstrated in this study, efficient and rapid concentration and purification of the generator-produced 68Ga can be performed on cation-exchangers if hydrochloric acid/acetone media are used. The procedure requires only 4 min and guarantees yields of 97% ± 1% of the initially eluted 68Ga, purified from 68Ge, Zn(II), Ti(IV), and even Fe(III).

The eluate processed according to the new protocol described in this study allows high labeling yields because of the increased chemical and radiochemical purities.

In addition, the significant removal of almost the entire 68Ge breakthrough on the cation-exchanger using the HCl/acetone systems fulfills the basic requirements relevant to the medical use of the radionuclide generator: Analyses of a possibly unexpected and critical breakthrough of 68Ge in each 68Ga fraction eluted (or in the final 68Ga-labeled pharmaceutical fraction) before the application of the 68Ga-labeled tracer do not belong to today’s routine procedures and are difficult to establish. The cation-exchange resin represents a guarantee for avoiding the transfer of this breakthrough to the labeling system or to the patient.

Furthermore, this procedure extends the useful life of the 68Ge/68Ga radionuclide generator systems. Even in cases in which the initial 68Ge breakthrough of a newly installed generator might be acceptable, this breakthrough might increase by an order of magnitude as a consequence of frequent elutions and definitely limits its long-term or high-volume application.

**Labeling**

Processing of the generator eluate provides excellent 68Ga purification, pH optimization ([H+] = 2.10−5 mol H+), as well as volume reduction, which guarantees high labeling yields and high specific activities for subsequent 68Ga-labeling reactions. The ~400 μL of acetone do not interfere. The procedure might even be modified, for example, by first evaporating the 400 μL of the 97.6% acetone/0.05N HCl fraction to dryness before adding the labeling precursor or by eluting 68Ga from the cation-exchange cartridge with a solution containing the labeling precursor.

**Specific Activity**

DOTA forms complexes with a ligand-to-metal ratio of 1:1. Because 1 nmol of 68Ga corresponds to 102.6 GBq activity, the theoretic (maximum) specific activity of 68Ga-DOTATOC is 102.6 GBq/nmol. Labeling in pure water provided specific activities of up to 50 MBq/nmol. Application of a buffer system allowed the optimization of reaction conditions—that is, decrease of volume (which in parallel increases concentration of the reagents). A specific activity of up to 450 MBq/nmol could be achieved. Nevertheless, this specific activity is still much lower than...
the theoretic value. Only about 0.5% of the DOTA-conjugated ligands are involved in complex formation with $^{68}$Ga(III).

A further increase in the specific activity requires decreasing the amount of ligand. However, handling of very small peptide quantities can be a critical factor for the labeling performance. Similar to Breeman et al. (8), we observed that the use of $<1-3$ nmol of the DOTA-conjugated peptide leads to its loss in the system. It decreases the reproducibility of the labeling procedure, which is especially problematic for routine production. Thus, increasing the initial $^{68}$Ga activity rather than decreasing the amount of ligand should be used as a strategy for preparation of labeled compounds with high specific activity. This again requires high efficiencies of $^{68}$Ga transfer from the generator to the labeling precursor.

Routine Clinical Application

The developed generator-associated eluate processing and labeling protocol were used to design a system for routine synthesis of $^{68}$Ga-DOTATOC, providing a safe kit-type labeling of DOTA-peptides even under conditions when an equipped radiochemical laboratory is not available. The time needed to process the generator eluate, to synthesize, and to purify $^{68}$Ga labeled DOTA-conjugated peptides, is <20 min. The chemical efficiencies related to each of the individual steps define a total yield of about 70% of $^{68}$Ga-DOTATOC in reference to the initially eluted $^{68}$Ga activity. This overall yield is higher than the yields of different batches, this process-inherent purification provides complete radiochemical purity. Additional quality control before the injection of the $^{68}$Ga-labeled compounds might be excluded in this case.

Content of Acetone

Acetone is classified as a solution with a negligible intravenous toxicity of 5,500 mg/kg (rat) (14). After 10 min, about 4.5 μg acetone remained in the reaction mixture. After purification of the $^{68}$Ga-labeled peptides on the RP18 cartridge, the overall acetone content in the final, injectable solution was $<0.5$ μg. Those numbers are far below the intravenous toxicity of acetone in rats or mice (LD$_{50}$ > 1 g/kg (14)).

Simultaneous Use of Several Generators

Due to the highly efficient purification and concentration performance of the post-processing protocol and due to the low hydrodynamic resistance of the generator system, it was possible to connect several generators in a cascade scheme. The eluent was piped through the first generator, which was connected to the eluent line of the next one. The second generator was connected directly to the microchromatography column. Alternatively, 2 generators can be eluted separately to one and the same cation-exchange column, which subsequently is processed as described.

A possible optimization scheme is presented graphically in Figure 2. It supposes that one half-life of $^{68}$Ge might be considered as a lower limit of the $^{68}$Ga activity required for radiopharmaceutical syntheses (solid line 1). To prolong the period of clinical generator use, its initial activity can be increased by a factor of 2 (i.e., 50-mCi vs. 25-mCi generators). Thus, 200% (dotted line 1") provides routine use for 2 $\cdot$ $t_{1/2}$. Alternatively, a cascade scheme supplements the amount of $^{68}$Ge, providing logistic advantages. An "old" 50% generator coupled with a fresh 100% generator results in 150% of overall $^{68}$Ge content (solid line 1 + 2). This 150% will provide enough $^{68}$Ga activity within 1.58 $\cdot$ $t_{1/2}$. Shelf-life of the first 100% generator in this case is approaching 1.3 $\cdot$ $t_{1/2}$. Administration of a third generator (solid line 1 + 2 + 3) increases this time up to 1.4 $\cdot$ $t_{1/2}$. This might be relevant in a context of routine clinical use, as it increases the efficacy of generator availability. Thus, application of several generators in a cascade scheme can be used to optimize the shelf life of the generators and to reduce costs.

![FIGURE 2. Cascade use of generators: Solid line (1) gives decay of a first, initial generator, which drops from 100% to 50% within 1 half-life of $^{68}$Ge. The 50% is considered here to determine the end of a generator usage. If, after this first half-life of 270 d, a second "fresh" generator of the same initial activity is added to the first, used one, the total activity of $^{68}$Ge is increased by a factor of 2 (i.e., 50-mCi vs. 25-mCi generators). This 150% will provide enough $^{68}$Ga activity within 1.58 $\cdot$ $t_{1/2}$.

Shelf-life of the first 100% generator in this case is approaching 1.3 $\cdot$ $t_{1/2}$; is achieved. This can easily be extended up to 1.4 $\cdot$ $t_{1/2}$. This might be relevant in a context of routine clinical use, as it increases the efficacy of generator availability. Thus, application of several generators in a cascade scheme can be used to optimize the shelf life of the generators and to reduce costs.

$^{68}$Ge/Ga GENERATOR POST-PROCESSING • Zhernosekov et al. 1747
CONCLUSION

A rapid, simple, and chemically efficient processing of generator-produced $^{68}$Ga was developed on the basis of cation-exchange chromatography in hydrochloric acid/acetone media. It successfully allows the purification and concentration of $^{68}$Ga. The procedure is described for a TiO$_2$-based commercial $^{68}$Ge/$^{68}$Ga radionuclide generator using 0.1N HCl as eluent. It might be adapted to other radionuclide generator types as well, whenever volume minimization and chemical and radiochemical purification are required before subsequent labeling reactions. For example, one of the initial attempts to design a $^{68}$Ge/$^{68}$Ga radionuclide generator was an anion-exchange resin eluted with 0.01N hydrofluorour acid solutions. It allowed high-purity separations due to the significant difference in the distribution coefficients of Ge(IV) and Ga(III) in the form of fluoro complexes (15). Its use, however, seemed to be rather complicated due to the problems arising from the amounts of HF in the $^{68}$Ga fraction eluted. The post-processing described in the present work would be perfectly compatible with this radiochemical concept as it absorbs $^{68}$Ga from 0.05N HF on the cation-exchange resin and provides, finally, an HF-free, 97.6% acetone/0.05N HCl fraction containing almost all of the $^{68}$Ga.

The post-processing of volumes and impurities is easily compatible with the synthesis of $^{68}$Ga-labeled compounds and their purification. The whole process guarantees safe preparation of injectable $^{68}$Ga-DOTATOC (or other $^{68}$Ga-labeled radiopharmaceuticals) for routine application and can be successfully used in the clinical environment. Automated versions of the processing are currently being developed.

Application of several generators in a cascade scheme can be used to optimize the shelf life of the generators and to reduce costs. The post-processing described here also provides a guarantee for chemical and radiochemical purification in this case.

ACKNOWLEDGMENTS

The authors thank the staff of the TRIGA Mainz and HMI BERII nuclear reactors for producing the radionuclide tracers. The close collaboration with the medical depart-

ments in Stuttgart and Bad Berka was a stimulus for our radiochemical investigations. This work was supported by the Deutsche Forschungsgemeinschaft (DFG Ro 985/18), the European Commission (FP6 NoE EMIL), and COST D38 is acknowledged.

REFERENCES