

## THE PURIFICATION AND THE QUALITY CONTROL OF $^{68}\text{Ga}$ ELUATES FROM $^{68}\text{Ge}/^{68}\text{Ga}$ GENERATOR\*

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*Abstract.* Today the majority of positron emission tomography (PET) studies are performed with F-18 radiopharmaceuticals requiring an on-site cyclotron or shipment from a site in close proximity to the place where the investigation is performed. Generator based radionuclides would allow easier availability and more flexibility in use.  $^{68}\text{Ge}/^{68}\text{Ga}$  generators provide cyclotron-independent access to PET radiopharmaceuticals.  $^{68}\text{Ga}$  is a short lived positron emitter (half-life 67.6 min) and is coming from his parent  $^{68}\text{Ge}$ , which have a half-life of 270.8 days. The 270 days half-life of the parent allows the use of the generator for a long period, potentially up to 1 year or even longer. The 67.6 minutes half-life of the  $^{68}\text{Ga}$  matches the pharmacokinetics of many peptides and other small molecules owing to rapid diffusion, localization at the target and fast blood clearance. The  $^{68}\text{Ga}$  solutions eluted from the generator are usually containing small amounts of other cations. Before the radiolabelling of peptides with it we have to purify the eluate. Because of the metallic impurities (Fe, Zn, breakthrough of Ge), the eluate has to be purified either on a cation exchange column, on an anion exchange column or both of them, combined. In this paper the results obtained after the purification on a cation exchange column, anion exchange column, both of them and fractioned elution will be presented.

Purification using anion resin and fractioned elution are feasible leading to a high quality eluate suitable for peptide radiolabelling.

*Key words:*  $^{68}\text{Ge}/^{68}\text{Ga}$  generator, purification, quality control, HPLC.

### 1. INTRODUCTION

The positron emission tomography is a non-invasive technique with a high resolution and sensitivity, offering a precise quantitative analysis. The radionuclides used in PET imaging are produced either in a cyclotron or in a generator. The obtaining of the PET radionuclides in a cyclotron is limited by the local existence of a cyclotron and by the high cost. The tracers based on the

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radioisotopes produced in generators of isotopes are cheaper, but their usage could be limited by the short half-lives of those radionuclides produced in this way.

The renewal of interest in the usage of the Ga-68 in PET imaging technique is due in large part by the innovation radiolabelling techniques using chelators or bifunctional agents. Thus, the Ga-68 is forming a stable complex with DOTA, allowing the radiolabelling of the peptides and other small molecules, resulting compounds with high specific activity. The short-lived positron emitter, 67.6 min, is compatible with the pharmacokinetics of many interest peptides in imaging diagnosis and therapy. The half-life of the parent radionuclide, 270.8 days, allows the usage of the  $^{68}\text{Ge}/^{68}\text{Ga}$  for at least one year, which makes him extremely practically and economically.

There are two naturally occurring isotopes of gallium:  $^{69}\text{Ga}$  (60.1% natural abundance) and  $^{71}\text{Ga}$  (39.9% natural abundance). For the medical interest, we can radiolabel the radiopharmaceuticals with three radioisotopes of gallium. Two of them,  $^{66}\text{Ga}$  ( $T_{1/2}=9,5$  h) and  $^{68}\text{Ga}$  ( $T_{1/2}=68$  min) are suitable for the PET imaging technique because of the  $\beta^+$  decay and  $^{67}\text{Ga}$  ( $T_{1/2}=78$  h) can be used for the SPECT imaging technique due to his gamma decay. With the aid of a generator system,  $^{68}\text{Ga}$  can be produced without the need of a cyclotron. The parent radionuclide,  $^{68}\text{Ge}$  is produced in accelerator through the nuclear reaction  $^{69}\text{Ga}(p,2n)^{68}\text{Ge}$ .  $^{68}\text{Ge}$  is disintegrating to  $^{68}\text{Ga}$  by exclusive electron capture, with a half-life of 270.8 days. The  $^{68}\text{Ga}$  decays through 89% by positronic emission, with the maximum energy 1.19 MeV and in 11% of the cases by electron capture to stable  $^{68}\text{Zn}$ . The half-life of  $^{68}\text{Ge}$  allows the production and the usage of the radiopharmaceuticals based on  $^{68}\text{Ga}$ .  $^{68}\text{Ga}$  is an ideal radiotracer positron emitter because of his non halogen and non volatile chemical properties, his half-life of 67.6 min which allow the manipulation of it in chemical synthesis, separations and purifications. The manual synthesis with radiotracers labeled with  $^{68}\text{Ga}$  lasts too long for the clinical applications, but the automation of the synthesis methods can significantly reduce this time. The major obstacles which have to be solved are:

- *the chemical form of  $^{68}\text{Ga}$  after the elution,*
- *the volume of elution,*
- *the contamination with other cations coming from the column,*
- *the possibility of contamination with  $^{68}\text{Ge}$ .*

The only stable chemical form in aqueous solution is the cation Ga(III), which can precipitate and hydrolysis at 3-7 pH in the insoluble form trihydroxide if the concentration exceeds the nanomolar level. In the presence of the stabilization agents, the precipitation can be avoided. At the physiologic pH, 7.4, the total solubility of Ga is bigger due to the formation, almost exclusive, of the ions  $[\text{Ga}(\text{OH})_4]^-$ . The Ga(III) cation is considered to be strong acid metal. It forms stable complexes with a large variety of ligands which contain oxygen, nitrogen or sulfur, as atoms donors. Thus, Ga(III) is suitable for the complexation with chelates, simple or conjugated with peptides or other macromolecules. The coordinative

chemistry of Ga(III) is defined by the number of coordination 6 and by the octahedral coordination sphere.

## 2. MATERIALS AND METHODS

- $^{68}\text{Ge}/^{68}\text{Ga}$  generator, 370 MBq, from iThemba, South-Africa
- Anion exchanger Dowex-1, 200-400 mesh
- Cation exchanger Dowex-50, 200-400 mesh

The  $^{68}\text{Ge}/^{68}\text{Ga}$  generator is a closed system, ready for use. We used a generator made by iThemba, from South Africa containing a tin dioxide column modified with polyethylene and also the tubing is made from polyethylene, without metallic parts (Fig. 1).  $^{68}\text{Ga}$  is continuously produced by the disintegration of the parent radionuclide  $^{68}\text{Ge}$  ( $T_{1/2}=270.8$  days). The generator is eluted with 0.6 M chloride acid (suprapur). The elution efficiency is at least 80% in 5 mL. The radioactivity given by the  $^{68}\text{Ge}$  what can occur in eluate is  $< 0.002\%$  to the reference date.



Fig. 1 –  $^{68}\text{Ge}/^{68}\text{Ga}$  generator.

## 3. RESULTS

### 3.1. CONCENTRATION AND PURIFICATION METHODS OF THE GA-68 ELUATE

The eluates intended for labeling purposes should be purified by cations competing to the chelator, coming from the column material, and have a high radioactive concentration. The chemical impurities from the eluate are radioactive germanium, which is coming from the potential breakthrough of it from the column and metallic ions: Fe(III), Cu(II), Sn(IV). We had tested a few concentration and

concentration/purification methods of the eluate based on the ions exchange technique.

### 3.2. FRACTIONED ELUTION

It is a concentration method of the eluate. The values graphically represented in Fig. 2 are the average of 14 experiments, in which we eluted the generator with 0.5 mL HCl 0.6M, up to 7 ml total volume. The fractions were collected in Eppendorf plastic tubes and they were measured using a calibrator, on the F-18 window. Simultaneously, the measurements were made using an installation with ionization chamber.

The data show that 80–92% of the eluted radioactivity was found in fractions 4, 5 and 6. This method leads to the concentration of the activity in a small volume, 1.5 mL, and thereby to the increasing of the radioactive concentration.

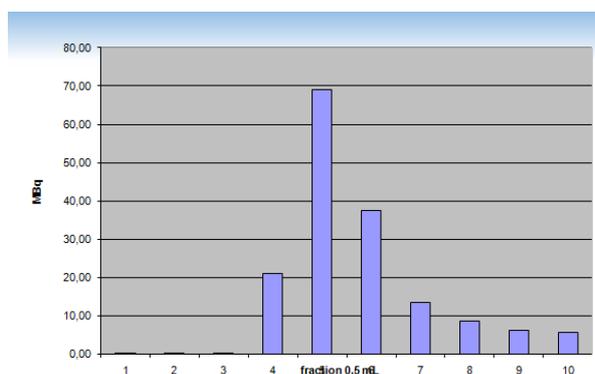


Fig. 2 – Graphical representation of the fractionated elution of the  $^{68}\text{Ge}/^{68}\text{Ga}$  generator.

### 3.3. ANION SEPARATION

This technique allows both, purification and concentration of the eluate. A mini-column (7 cm length, 0.7 cm diameter) was prepared, containing 50 mg anion exchange resin Dowex-1, 200–400 mesh. The column was pretreated and brought in  $\text{H}^+$  form. The generator was eluted with 7 mL HCl 1 N and 5.5 mL HCl 12 N were added, obtaining a solution 5.5–6 N. At this normality, gallium is found in  $[\text{GaCl}_4]^-$  form (Fig. 3, HPLC), pH 0.5; it is fully retained on the anion exchange column while the metallic cations are passing through the column and collected in plastic tubes. The recovery of gallium from the column is made with water. As one can see in Fig. 4, gallium is recovered in 1 mL water, having 84–90% from the loaded radioactivity at the column top (results of 4 experiments). The subsequent

washes had an activity at the background level. The radiochemical purity of the radioactive fraction was tested through HPLC and the radiochromatogram is presented in Fig. 5. We used acetonitrile and water as mobile phases, both acidified with 0.1% TFA; the stationary phase was a C-18 column, Nucleosil, 250 × 4.6 mm. The retention time for the gallium was around 3.5 min. The radiochemical purity was higher than 98%. The time for this experiment was about 30 minutes.

We consider this concentration and purification method optimal, with good values for both of the critical parameters: the process time and the recovery percentage of  $^{68}\text{Ga}$ .

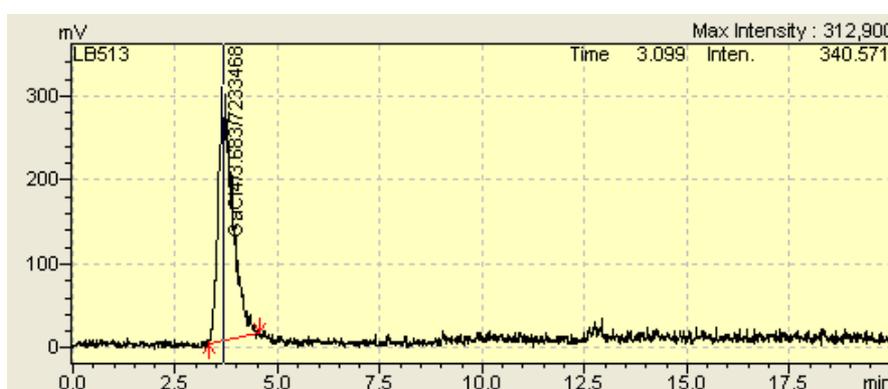


Fig. 3 – HPLC radiochromatogram of eluate at pH 0.5 and in  $[\text{GaCl}_4]$  form.

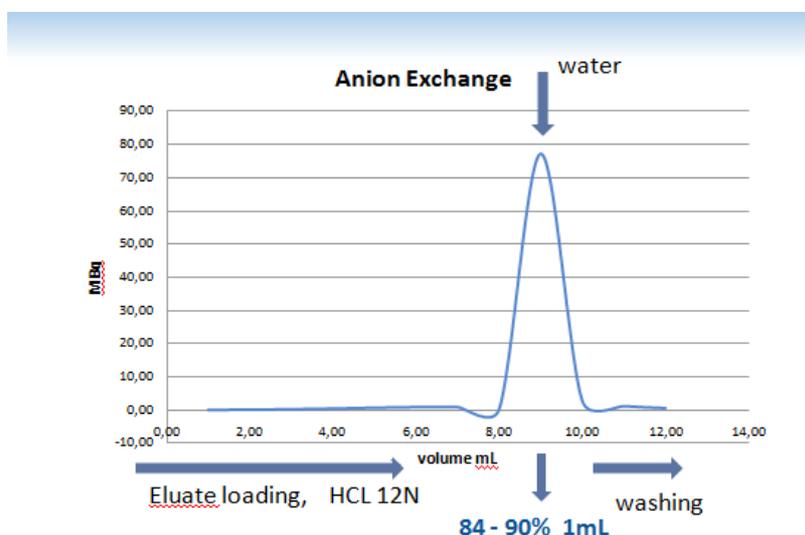


Fig. 4 – Graphical representation of the concentration and purification process of the eluate from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator using anion exchange column.

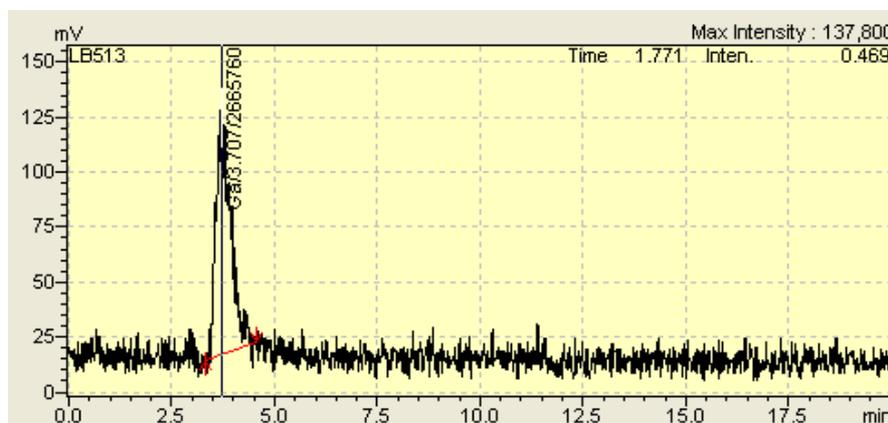


Fig. 5 – HPLC radiochromatogram of eluate purified on an anion exchange column.

### 3.4. CATION SEPARATION

This technique, as previously, allows both purification and concentration of the eluate. A mini-column (7 cm length, 0.7 cm diameter) containing of 50 mg cation exchange resin Dowex-50, 200-400 mesh was prepared. The column was pretreated and brought in  $\text{Cl}^-$  form. The generator was eluted with 7 mL HCl 1 N and gallium was found to be in the ionic form,  $\text{Ga}^{3+}$  (Fig. 6, HPLC), pH 0.5. The eluate was loaded on the top of the column; gallium was fully retained on the cation exchange column, while germanium passed through and was collected in the first fractions. The column was successively washed with 2 mL acetone/chloride acid 0.1 M 80/20, then with 3 fractions of each 0.4 ml acetone/chloride acid 0.5 N 98/2, 1 mL chloride acid 4 N and 2 mL water. The radioactivity of the collected fractions was measured. The recovery of gallium from the column is made in the first two fractions with acetone/chloride acid 0.5 N 98/2 (total volume 0.8 mL). As we represented in Fig. 7, gallium is recovered in variable proportion, 20–67% from the loaded activity on the column (results of 8 experiments). In some cases, the biggest percentage from the loaded activity was found in the fraction eluted with HCl 4 N. The subsequent washes had a radioactivity at the background level. The radiochemical purity of the radioactive fraction was tested through HPLC and the radiochromatogram is represented in Fig. 8. The HPLC experiments were done using the same conditions presented for anion separation experiments. The retention time for the gallium was around 3.5 min. The time for this experiment was about 35 minutes.

We consider this concentration and purification method non-reproducible because of the wide range of the recovered radioactivity.

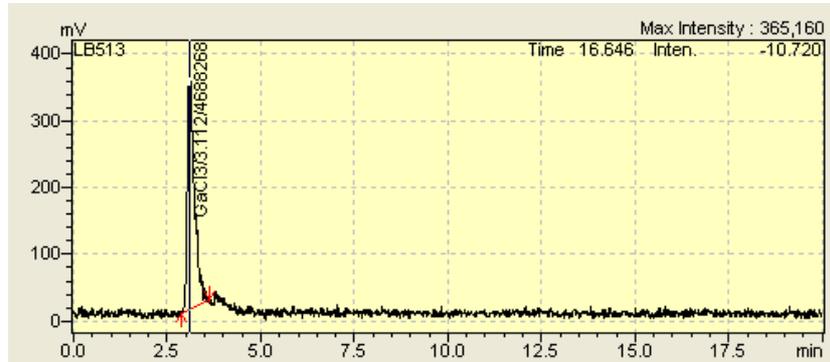


Fig. 6 – HPLC radiochromatogram of eluate at pH 0.5 in  $\text{GaCl}_3$  form.

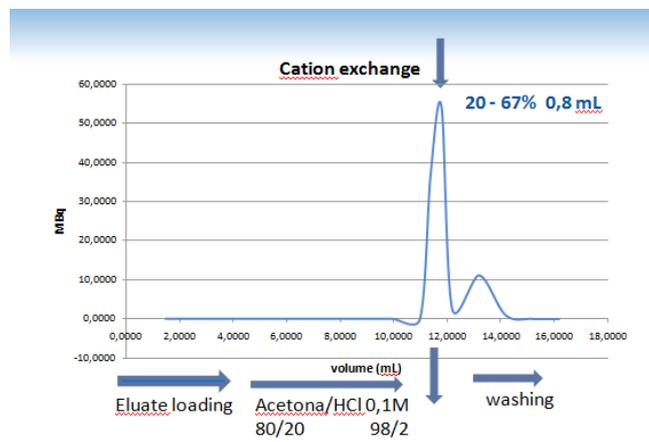


Fig. 7 – Graphical representation of the concentration and purification process of the eluate from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator using cation exchange column.

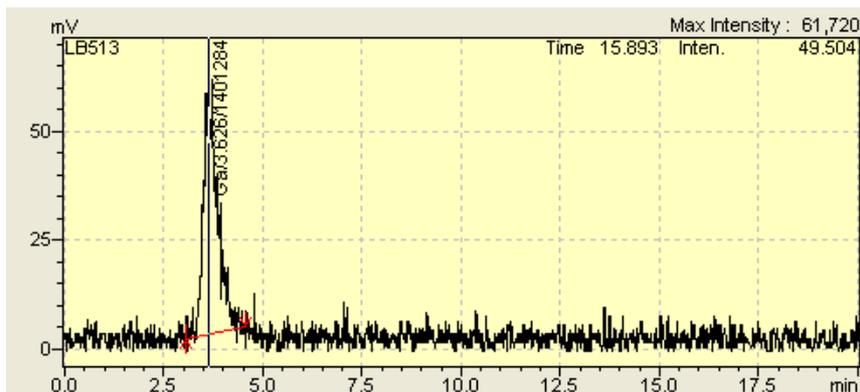


Fig. 8 – HPLC radiochromatogram of eluate purified on a cation exchange column.

### 3.5. ANION AND CATION SEPARATION

For a better separation of the metallic ions we combined the two purification methods. Initially, the generator was eluted with 5 mL HCl 0.6 N, the eluate was loaded on the cation exchange column and gallium was extracted from the column with HCl 4 N in 1.5 mL. This solution was then loaded on the anion exchange column and it was recovered in 1 mL water (Fig. 9, HPLC). The process is described in Fig. 10. The radioactivity percentage found in the final volume, 1 mL, was up to 95% from the loaded activity on the first column, but the necessary time for this process was too long, 40–60 minutes (almost a half-life of  $^{68}\text{Ga}$ ).

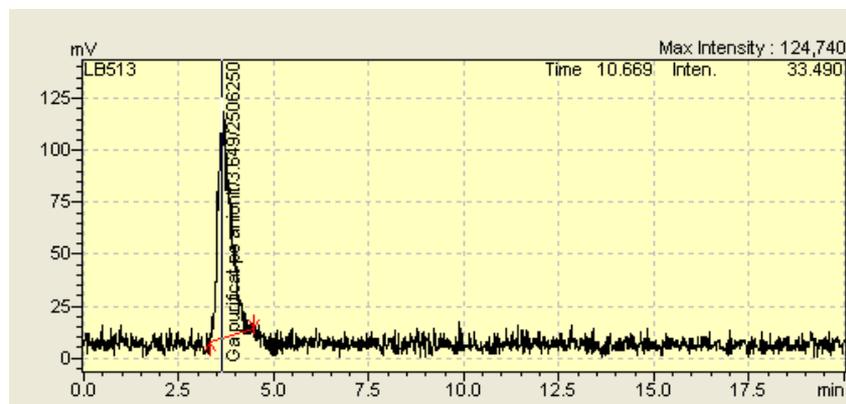


Fig. 9 – HPLC radiochromatogram of eluate purified with the combined method.

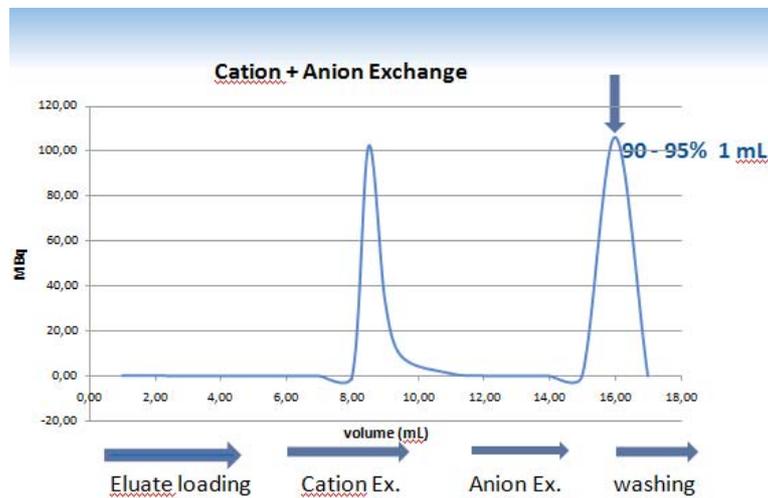


Fig. 10 – Graphical representation of the concentration and purification process of the eluate from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator using the combined method.

#### 4. CONCLUSIONS

As a result of this study we conclude:

- The anion separation represents an optimal concentration and purification method: the processing time of 30 min and the recovery percentage of Ga-68, up to 90%;
- The cation separation represents a non-reproducible method for concentration and purification of the eluates;
- The combined method offers a good concentration and purification of the eluate, but the necessary time for this process was almost a half-life of Ga-68.
- The eluate from  $^{68}\text{Ge}/^{68}\text{Ga}$  generator with tin dioxide column has to be processed before the radiolabelling procedures, but fractionated elution could also be used for synthesis, when short reaction time is needed and further purification is envisaged.

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