Optimization of automated radiosynthesis of gallium-68-labeled PSMA11 with two 68Ge/68Ga generators: fractional elution or prepurification?

Flore Durieux (flore.durieux@chu-lille.fr)
Centre Hospitalier Universitaire de Lille

Bérengère Dekyndt
Centre Hospitalier Universitaire de Lille

Jean-François Legrand
Centre Hospitalier Universitaire de Lille

Antoine Rogeau
Centre Hospitalier Universitaire de Lille

Emmanuel Malek
Centre Hospitalier de Valenciennes

Franck Semah
Centre Hospitalier Universitaire de Lille

Pascal Odou
Centre Hospitalier Universitaire de Lille

Research Article

Keywords: Radiopharmacy, gallium 68, double elution

Posted Date: April 6th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2727479/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Prostate cancer is one of the most common forms of cancer in men. An imaging technique for its diagnosis is $^{68}$Ga-prostate specific membrane antigen ($^{68}$Ga-PSMA-11) positron emission tomography (PET). Gallium 68 ($^{68}$Ga) is typically obtained using a germanium 68/gallium 68 ($^{68}$Ge/$^{68}$Ga) generator, allowing for the diagnostic drug to be made readily available in the nuclear medicine department through elution. To meet the increasing demand for $^{68}$Ga labeled peptides and to reduce the cost of radiosynthesis, it is therefore necessary to optimize the elution process of $^{68}$Ge/$^{68}$Ga generators. This study aims to identify the most effective approach for optimizing radiosynthesis using double elution in parallel of two $^{68}$Ge/$^{68}$Ga generators. Two methods have been tested: one using prepurification, and the other using fractionated elution.

Results

Five synthesis sequences were conducted using each method. The mean labeling yields for double elution with prepurification were $45.8 \pm 29.4$ (mean $\pm$ standard deviation) and none met the required criteria. The mean labeling yields for the fractionated double elution were $97.5 \pm 1.9$ (mean $\pm$ standard deviation) meeting the criteria, significantly superior to the prepurification method ($p=0.012$), and similar to those of simple elution. There was no significant difference in the elution yields of both methods.

Conclusions

This study showed that fractionated double elution from $^{68}$Ge/$^{68}$Ga generators produced a significantly higher labeling yield than double elution with prepurification, resulting in a larger activity recovered by radiosynthesis, and thereby allowing for more diagnostic tests to be performed. Additionally, this method does not add complexity or synthesis time compared to simple elution labeling, and could also be applied to other $^{68}$Ga labeled peptides.

Background

Prostate cancer is the 4th most common form of cancer in Europe and the most common in men (1). According to the European Society for Medical Oncology (ESMO) guidelines, the diagnosis of high-risk forms relies on imaging techniques including magnetic resonance imaging (MRI), $[^{18}F]$-fluorocholine positron emission tomography (PET), and $[^{68}Ga]$-prostate specific membrane antigen ($^{68}$Ga-PSMA-11) PET (2). In France, $^{68}$Ga-PSMA-11 is a radiopharmaceutical drug with compassionate access for patients with biologically recurrent prostate cancer, defined as a re-increase in serum prostate specific antigen (PSA) concentration (3). This radiopharmaceutical drug has high sensitivity and excellent specificity for the diagnosis of prostate cancer and is increasingly used in routine practice (4).
Gallium 68 can be obtained via cyclotron production with a \(^{68}\text{Zn}\) target solution or from elution of a germanium \(^{68}\text{Ge}/^{68}\text{Ga}\) generator. These generators can be eluted on-site, making radiolabeled PSMA available free from industrial production constraints, several times a day, and up to one year due to the \(^{68}\text{Ge}\) period of 271 days. However, they come with limitations which are their cost and a limited activity (only 1850 MBq available in France). Because of the short half-life of \(^{68}\text{Ga}\) (68 min), the dosage of \(^{68}\text{Ga-PSMA-11}\) (2 MBq/kg), and the time between injections (25 minutes), not many patients can be injected per elution. To address the growing demand for \(^{68}\text{Ga-PSMA-11}\) and reduce the cost of the radiopharmaceutical / patient, optimization of radiosynthesis has been implemented. Two possible options are to elute a generator calibrated with high activity or to elute two generators in parallel (5).

In the present study, we investigate the radiosynthesis optimization of \(^{68}\text{Ga-PSMA-11}\) based on the double elution in parallel of two \(^{68}\text{Ge}/^{68}\text{Ga}\) generators with calibration dates six months apart. There is limited literature on the subject, and this double elution is expected to increase the activity of radiosynthesis and ensure a more consistent supply of \(^{68}\text{Ga-PSMA}\), which would improve the flow of PET exams over time.

Our objective was to compare two methods in order to determine the most suitable, \textit{i.e.} the one that guarantees the best synthesis yield, following the Good Manufacturing Practices for Preparations:

- Double elution with prepurification to concentrate the gallium 68
- Double fractional elution to extract the most concentrated fraction in activity of the two eluates

**Methods**

The equipment used for each radiosynthesis method is detailed in Table 1 and the preparation steps are summarized below.
Table 1
Material used for radiosynthesis with prepurification and fractional double elution.

<table>
<thead>
<tr>
<th>Double elution with prepurification</th>
<th>Double fractional elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High energy shielded enclose: H700hotcell, Trasis® (Ans, Belgium)</td>
<td></td>
</tr>
<tr>
<td>MiniAIO® synthesis module, Trasis®</td>
<td></td>
</tr>
<tr>
<td>Peptide PSMA-11, Iason® (Graz-Seiersberg, Austria)</td>
<td></td>
</tr>
<tr>
<td>Generators $^{68}$Ge/$^{68}$Ga GalliaPharm® Eckert &amp; Ziegler (Berlin, Germany)</td>
<td></td>
</tr>
<tr>
<td>Elution solvent: 1 M hydrochloric acid Eckert &amp; Ziegler</td>
<td></td>
</tr>
<tr>
<td>Acetate buffer 1 M, Trasis®</td>
<td>Acetate buffer 0.7 M, prepared extemporaneously</td>
</tr>
<tr>
<td>Cassette with prepurification ® ref: S10886</td>
<td>Cassette without prepurification, Trasis® (Ref: S7577)</td>
</tr>
<tr>
<td>Trasis®</td>
<td></td>
</tr>
<tr>
<td>C18 prepurification cartridge, Oasis®</td>
<td></td>
</tr>
<tr>
<td>Suprapur® NaCl/HCl 30%</td>
<td></td>
</tr>
<tr>
<td>HLB cartridge</td>
<td></td>
</tr>
<tr>
<td>NaCl 0.9%</td>
<td></td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td></td>
</tr>
</tbody>
</table>

**The double elution with prepurification method**

- Cassette with prepurification is placed on the MiniAIO® synthesizer
- 10 µg of PSMA-11 are reconstituted with 3 mL of sodium acetate buffer from Trasis® and then transferred into the reaction vial (step 1 in Fig. 1)
- Elution of the two $^{68}$Ge/$^{68}$Ga generators is performed in parallel, by 5 mL of HCl 1M (2 mL/min) each. A female Luer lug style tee and a ventilated filter are positioned at the outlet of the two generators (step 2)
- $^{68}$Ga solution is transferred to the prepurification cartridge which is eluted with 5 mL of a Suprapur® NaCl/HCl 30% mixture (step 3)
- Labeling reaction is performed at 95 ± 5°C during 6.5 min (step 4)
- The solution is loaded onto an HLB cartridge to retain the $^{68}$Ga-PSMA-11. This cartridge is rinsed with 0.9% NaCl. The free $^{68}$Ga is evacuated in waste (steps 5 and 6)
- Elution of the cartridge with 0.7 mL of absolute ethanol, recovering the $^{68}$Ga-PSMA-11 (step 7)
- Transfer of the solution to the final vial
- Formulation with 9.3 mL of 0.9% NaCl. Volume of the final product is 10 mL (step 8)
The double fractional elution method

- Cassette without prepurification is placed on the MiniAIO® synthesizer.
- 10 µg of PSMA-11 is reconstituted with 1 mL of 0.7 M sodium acetate solution, prepared and filtered in radiopharmacy under laminar flow (ISO 5), and then transferred into the reaction vial (step 1 in Fig. 3).
- Elution of the two $^{68}$Ge/$^{68}$Ga generators is performed in parallel, by 5 mL of HCl 1M (2 mL/min) each. A T device and a ventilated filter are positioned at the outlet of the two generators (step 2).
- The first 3 mL, i.e. 1.5 mL for each generator and the last 1 mL, i.e. 0.5 mL for each generator, of the elution are eliminated in waste (steps 3a and 3b). These volumes were established according to a study (5) showing that fractionation of the elution from the $^{68}$Ge/$^{68}$Ga generators resulted in a higher activity volume. The following diagram from the study shows this result (Fig. 2).
- 6 mL of the elution, the most active fraction, are transferred to the reaction vial containing the peptide in solution in acetate buffer (step 4)
- Labeling reaction is performed at 95 ± 5°C for 6.5 min (step 5)
- The solution is loaded onto a C18 cartridge (SEP-Pak) which retains the $^{68}$Ga-PSMA-11. The cartridge is rinsed with 0.9% NaCl (step 6)
- Elution of the cartridge with 0.7 ml of absolute ethanol removing the $^{68}$Ga-PSMA-11 transferred to the final vial. The final product is then diluted with 9.3 mL of 0.9% NaCl (steps 7 and 8)

The acceptance criteria

For each synthesis procedure, quality controls were carried out:

- Visual examination of organoleptic character: the product must be clear and colorless
- pH measured with a pH strip must be between 4 and 8
- Radiochemical purity (RCP), measured by two methods:

High performance liquid chromatography (HPLC) with C18 column Waters® 250 x 4.6 mm, flow: 1 ml/min, with solvent gradient Acetonitril/TFA 0,1% and water/TFA 0,1%. The programming is 00:00–3:00 min: 97% A -3% B; 06:00–9:00 min: 0% A -100% B et de 12:00–15:00 97% A -3% B. The retention time is 3.7 min for free $^{68}$Ga and 8.3 min for $^{68}$Ga-PSMA-11. The addition of the stereoisomers is at least 95% of the total radioactivity due to $^{68}$Ga.

Thin layer chromatography (TLC) with ITLC-SG plates, where the retention factor of $^{68}$Ga-PSMA-11 is 0.8-1, that of $^{68}$Ga 3 + is 0-0.1 and that of the $^{68}$Ga colloid is 0.7. No less than 95% of the total radioactivity is due to $^{68}$Ga-PSMA-11.
• Bacterial endotoxins, measured by a kinetic and chromogenic LAL method (Endosafe®, Charles River), the preparation is diluted to the 10th and deposited on PTS-10 cassettes with a sensitivity of 0.01EU/ML - PTS2001. The expected result is a bacterial endotoxin content lower than 15.1 IU/mL

• Radiolabeling yield, measured with an ISOMED2010® dose calibrator, which is expected to be higher than 80%

• Elution yields were calculated. The manufacturer specification of elution yield is > 62%, but the prepurification/fractioning steps tend to decrease this yield (about 10%) and therefore we set the specification to elution yield > 55%.

**Statistical tests**

A Mann-Whitney test was performed to compare elution and labeling yields as well as the overall radiosynthesis yields (multiplication of the elution and labeling yields) of the two methods.

**Results**

A total of 10 radiosynthesis sequences were performed: 5 with prepurification and 5 with fractionated elution. All yields (elution and labeling) were measured at the time of elution.

The labeling yields of the elution with prepurification were between 6 and 74% and therefore did not comply with the expected yields. Furthermore, the radiolabeling success was inconsistent (mean of labeling yield: 45,8 ± 29,4%). For the third radiosynthesis, the quality controls were not compliant with a TLC-assessed RCP = 71,8%. For this radiosynthesis, HPLC was not performed because the TLC was already not compliant. The elution yields were compliant to specifications (63,8 ± 4,8%). Radiosynthesis results are detailed in Table 2.

Table 2: Results of double elution radiosynthesis with prepurification

<table>
<thead>
<tr>
<th>Radiosynthesis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution yield (%)</td>
<td>62.7</td>
<td>71.7</td>
<td>64.5</td>
<td>59.4</td>
<td>60.7</td>
<td>63.8 ± 4.8</td>
</tr>
<tr>
<td>Labeling yield (%)</td>
<td>73.0</td>
<td>54.3</td>
<td>6.4</td>
<td>24.4</td>
<td>69.0</td>
<td>45.8 ± 29.4</td>
</tr>
<tr>
<td>Elution yield * Labeling yield (%)</td>
<td>46.3</td>
<td>38.9</td>
<td>4.1</td>
<td>14.5</td>
<td>42.4</td>
<td>29.2 ± 18.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality control</th>
<th>pH</th>
<th>6</th>
<th>5</th>
<th>6</th>
<th>5</th>
<th>5.8 ± 0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>51Ga free rate in TLC (%)</td>
<td>1.7</td>
<td>4.6</td>
<td>28.2</td>
<td>0.1</td>
<td>1.0</td>
<td>7.1 ± 11.9</td>
</tr>
<tr>
<td>51Ga free rate in HPLC (%)</td>
<td>3.3</td>
<td>6.7</td>
<td>-</td>
<td>4.6</td>
<td>4.6</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>Radiochemical purity (%)</td>
<td>96.7</td>
<td>93.3</td>
<td>71.8</td>
<td>95.4</td>
<td>96.4</td>
<td>90.5 ± 10.6</td>
</tr>
<tr>
<td>Endotoxins (UI/mL)</td>
<td>&lt; 15.1</td>
<td>&lt; 15.1</td>
<td>&lt; 15.1</td>
<td>&lt; 15.1</td>
<td>&lt; 15.1</td>
<td>&lt; 15.1</td>
</tr>
</tbody>
</table>

As for double fractional elution, yield and quality control requirements were met for all sequences. The labeling yields were between 94% and 99%, as shown in Table 3, and the elution yields were compliant to specifications (59,4 ± 2,2%)
There was no significant difference between the elution yields of both methods ($p = 0.151$), but there was a significant difference in labeling yields between the prepurification and the fractionated elution methods ($p = 0.012$), in favor of the latter. Regarding overall radiosynthesis yields, the difference was also significant ($p = 0.008$).

Table 3: Results of fractionated double elution radiosynthesis

<table>
<thead>
<tr>
<th>Radiosynthesis</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution yield (%)</td>
<td>61.2</td>
<td>56.1</td>
<td>59.5</td>
<td>61.6</td>
<td>58.8</td>
<td>59.4 ± 2.2</td>
</tr>
<tr>
<td>Labeling yield (%)</td>
<td>97.1</td>
<td>98.1</td>
<td>94.5</td>
<td>99.0</td>
<td>99.0</td>
<td>97.5 ± 1.9</td>
</tr>
<tr>
<td>Elution yield * Labeling yield (%)</td>
<td>59.4</td>
<td>55.0</td>
<td>56.2</td>
<td>61.0</td>
<td>58.2</td>
<td>58 ± 2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality control</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>$^{68}$Ga free rate in TLC (%)</td>
</tr>
<tr>
<td>$^{68}$Ga free rate in HPLC (%)</td>
</tr>
<tr>
<td>Radiochemical purity (%)</td>
</tr>
<tr>
<td>Endotoxins (IU/mL)</td>
</tr>
</tbody>
</table>

Discussion

In this study, we show that double elution with prepurification led to insufficient and greatly varying radiolabeling yield of $^{68}$Ga-PSMA-11, whereas fractionated double elution resulted in consistent and higher labeling/overall yield.

There are several potential explanations for the results of the synthesis with elution prepurification. One possibility is that there were issues with pH. pH is a critical factor in the radiosynthesis of $^{68}$Ga-PSMA-11, and a too acidic pH can result in the protonation of the chelator. On the other hand, if the pH is too basic, it can lead to the formation of insoluble $Ga(OH)_3$. In this method, 5 mL of an HCl/NaCl mixture were added to 3 mL of acetate buffer. In comparison, in the double fractionation elution method, 6 mL of $^{68}$Ga in HCl 0.1M are added to 1 mL of acetate buffer. As the reaction medium is different, the pH may vary. Unfortunately, the pH at this step is not an “in-process” checkpoint leaving doubt as to the role of pH in the synthesis failure (6)(7).

Another hypothesis might have been the presence of impurities in the reaction vial. Metallic impurities can be caused by degradation of the generator column or the purification process. Ferric impurities may compete with $^{68}$Ga, preventing complexation with the chelator and thereby decreasing the yield. However, this hypothesis was effectively ruled out since single elution was performed and quality checks/yields met requirements (see supplementary information, Table 1) (8)(9).

As a result of this, we started to question the overall preparation process. Interestingly, Reverchon et al (10) showed that the best conditions for the preparation of $^{68}$Ga-PSMA-11 may involve no purification and no heating, which significantly reduced the preparation time. Under these conditions, quality controls
showed a RCP of over 99% and a radiolabeled yield of over 99%. This approach, however, creates other issues. Removing the purification step leads to a larger elution volume (10 mL), and requires a complete modification of the radiosynthesis process including changes in acetate buffer volume, heating profile, and volume activity for labeling.

Finally, all fractional elution tests produced results within specifications. Fractional elution appears to be the method of choice for $^{68}$Ga-PSMA-11 radiosynthesis for several reasons. Firstly, as previously mentioned, fractional elution allows for the removal of the eluate portion containing metallic impurities, while retaining the part with the highest activity for radiolabeling with an almost identical volume to that of simple elution. Although the elution yields may be slightly lower, the labeling yields are significantly higher. Secondly, the preparation process is simplified as there is no longer a need for prepurification, making it similar to simple elution which shows high labeling yields. Lastly, the simplified preparation process saves time and therefore results in higher activity.

**Conclusion**

The literature on fractional elution for PSMA-11 radiolabeling remains limited, and our study demonstrates that this method applied on a $^{68}$Ge/$^{68}$Ga generator can optimize radiosynthesis. Fractional elution allows for a higher activity product enabling more patients to benefit from $^{68}$Ga-PSMA-11 PET for diagnosis, while decreasing the frequency of radiosynthesis per week. This optimization can help to plan for the anticipated huge increase in $^{68}$Ga-PSMA-11 PET-scans. Additionally, double fractional elution could also be applied to the labeling of other peptides such as edotreotide.

**Abbreviations**

$^{68}$Ga-PSMA-11
[68Ga]-prostate specific membrane antigen
$^{68}$Ge
Germanium 68
$^{68}$Ga
Gallium 68
HPLC
High performance liquid chromatography
PET
Positron emission tomography
PSA
Prostate specific antigen
RCP
Radiochemical purity
TLC
Thin layer chromatography

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

Funding

Nuclear medicine department, CHU Lille, F-59000 Lille, France

Authors’ contributions

FD, BD and JFL performed the radiosynthesis and worked on preparation method. BD, JFL, AR, EM, FS, PO reviewed the manuscript. All authors approved the final manuscript.

Acknowledgements

Not applicable.

References


Figures
Figure 1

Overview of the double elution with prepurification
Figure 2

Profile of unfractionated 68Ga elutions. From Boukhlef et al (5).

Figure 3

Overview of the double fractional elution