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The influence of generator eluate in radiolabeling PSMA-11 kit with ^{68}Ga

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ABSTRACT

Gaining prominence in clinical practice, the ^{68}Ga, positron emitter radionuclide easily obtained by ^{68}Ge/^{68}Ga generator elution, has shown potential and excellent quality on radiolabeling of peptide for use in positron emission tomography (PET), in particular urea-based inhibitor peptides, directed to the prostate-specific membrane receptor (PSMA). Previous studies with the PSMA linked to the chelator HBED-CC (PSMA-11) radiolabeled with ^{68}Ga showed high contrast PET/CT images to evaluate recurrence and metastasis of prostate cancer, becoming an important imaging agent in the clinical routine. This work intended to evaluate the influence of the quality of the ^{68}Ge/^{68}Ga generator eluate in direct labelling of PSMA-11 with ^{68}Ga, assisting in the development of kit for prompt radiolabeling. It was evaluated the $^{68}GaCl_3$ eluate from ^{68}Ge/^{68}Ga non-GMP generator (manufacturer A) and ^{68}Ge/^{68}Ga GMP generator (manufacturer B), both commercially available. To evaluate the influence of the ^{68}Ga eluate on radiochemical yield of the preparations, the radiochemical purity was determined by thin layer chromatography and HPLC. The radiolabeling with non-GMP generator eluate was made with and without preliminary purification of the 68 gallium chloride eluate, employing cationic purification columns. The results showed higher radiochemical yield with the $^{68}GaCl_3$ eluate from the ^{68}Ge/^{68}Ga GMP generator, obtaining the radiolabeled product more easily and speed to clinical practice, without preliminary purification, as opposed to the use of non-GMP ^{68}Ge/^{68}Ga generator which required preliminary purification of the $^{68}GaCl_3$ eluate to promote satisfactory radiochemical purity results.

***Keywords*: prostate cancer, radiolabeling, radiopharmacy.**

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1. INTRODUCTION

The according with data compiled by the World Health Organization [1] for the year 2018, cancer has become the main cause of the decrease in the life expectancy of the world, knocking down the numbers of deaths from coronary heart disease and stroke. The causes are still debatable, however, according to studies conducted, the risk factors depend on regional socioeconomic development [1].

In the ranking, prostate cancer appears second by incidence in men, presenting 1.276,106 new cases of pathology in 2018, according to data collected by GLOBOCAN [1].

For the years 2018 and 2019, data raised by INCA [2] suggest the incidence of 600 thousand new cases of cancer in Brazil for each year, putting prostate cancer among the most incident cancers.

With greater occurrence in men of the old age, the prostate cancer still has no definite causes. Anatomically, the prostate is a gland located in the lower part of the bladder, composed of a fibromuscular stromal network, vascular endothelium, immune cells and several other cell types organized rearrangement in an epithelium. According to this heterogeneity of the gland, studies are difficult when one wants to discover the causes of pathology [3].

Wishing diagnostic methods with greater specificity and sensitivity, the diagnostic imaging tests used in nuclear medicine with the administration of radiopharmaceuticals present themselves with great advances, since the clinical practice is confronted with controversial results in the tests of touch and serum dosage of PSA, generating false-negative results, which decreases the reliability of these results and delays the early diagnosis [4].

During the development of prostate cancer, occurs an overexpression of PSMA (prostate specific membrane antigen), a type II transmembrane protein, present in 95% of cases of prostate câncer [5].

The continuous internalization through migration between the cell membrane and the cytoplasm, makes the PSMA a therapeutic target, when in the presence of binding molecules [5].

Owing this characteristic, radiotracers targeting PSMA are being studied for acquisition of diagnostic imaging of prostate cancer in PET equipment, in particular the PSMA linked to the bifunctional chelator HBED-CC (PSMA-11) radiolabeling with ^{68}Ga [5].

Easily obtained by elution of ^{68}Ge/^{68}Ga generators and with a physical half-life of approximately 68 minutes, the ^{68}Ga radionuclide has been widely used in nuclear medicine for imaging diagnostic tests on PET equipment. Obtaining the eluate from the generators facilitates clinical routine and assists in delivering the product to clinics away from centers producing cyclotron radiopharmaceuticals [6].

The eluate from the ^{68}Ge/^{68}Ga generator has metallic impurities that, if not removed, can compete with ^{68}Ga in solution decreasing the percentage of radiochemical purity of the final product. In its composition the ^{68}Ge may be immobilized on an alumina column, tin or titanium dioxide, being able to elute ^{68}Ga with 0.1 M HCl, 0.05 M or 1 M, depending on the generator model used [7].

This work aims to study the influence of commercially available ^{68}Ge/^{68}Ga generators eluates for the development of a radiolabelled PSMA-HBED-CC kit with ^{68}Ga, from preliminary labeling conditions.

2. MATERIALS AND METHODS

2.1. Previous study of radiolabeling

The assays and parameters of radiolabeling were initially based on the kit developed by POLATOM (Poland), for radiolabeling of PSMA-HBED-CC with ^{68}Ga, containing 20 μg of the PSMA-HBED-CC peptide and sodium acetate in the formulation. The radiolabeling instruction of the kit provides the use of 5 ml of $^{68}GaCl_3$ and 10 minutes at 90ºC. The radiolabeling conditions were evaluated for different elutions of ^{68}Ge/^{68}Ga generators, being of manufacturer A and manufacturer B, corresponding respectively, to a non-GMP ^{68}Ge/^{68}Ga generator, with a titanium oxide column, elute with HCl 0.1 M and a ^{68}Ge/^{68}Ga GMP generator with silica column, eluted with 0.05M HCl.

In this work, the radiolabeling buffer was evaluated. The sodium acetate buffer was prepared in different molar concentrations evaluated against different volumes of solution of 0.1 M HCl and 0.05 M HCl, employed in the elution of commercially available ^{68}Ga generators.

For all experiments developed, ^{68}Ge/^{68}Ga generator from manufacturer A was eluted with 0.1M HCl and the generator of manufacturer B was eluted with 0.05M HCl to obtain 5 mL of $^{68}GaCl_3$.

2.2. Chromatographic system choice for radiochemical purity test

The chromatographic profile of $^{68}GaCl_3$ and the radiolabeling mixture was studied in two systems: (1) Thin-film chromatography with mobile phase citrate buffer: citric acid 1M (v/v), pH 5, and mobile phase methanol : ammonium acetate 1M (v/v), pH 8,5 in chromatographic supports of ITLC-SG (Instant Thin Layer Chromatography with silica gel) and TLC-SG (Thin Layer Chromatography with silica gel) and (2) High Performance Liquid Chromatography (HPLC) - Mobile phase A: trifluoroacetic acid, water for chromatography (1:999 v/v). Mobile phase B: trifluoroacetic acid, acetonitrile (1:999 v/v). Stable phase column of octadecylsilyl silica gel for chromatography, 3 mm in diameter and 0,15 m wide. The solvent concentration gradient is described in Table 1 [8].

Table 1: Solvent concentration gradient described by the European pharmacopoeia for ^{68}Ga-PSMA-11

Tempo (min)	Fase móvel A (v/v)	Fase móvel B (v/v)
0 – 0.5	95	5
0.5 - 10	95 - 60	5 - 40
10 - 11	60 - 95	40 - 5
11 - 16	95	5

2.3. Non-automated radiolabeling of PSMA-HBED-CC with $^{68}GaCl_3$ eluted from ^{68}Ge/^{68}Ga generator of manufacturer A without prior purification

Six labels were performed employing $^{68}GaCl_3$ without prior purification under the conditions described above. The range of activity employed in the radiolabelling was 333 to 370 MBq (9 to 10 mCi).

2.4. Non-automated radiolabeling of PSMA-HBED-CC with $^{68}GaCl_3$ eluted from $^{68}Ge/^{68}Ga$ generator of manufacturer A with previously purified

Radiolabeling was performed with prior purification of the eluate from the $^{68}Ge/^{68}Ga$ generator of manufacturer A in a cationic cartridge identical to that used in the automated modules for the synthesis of ^{68}Ga-DOTATATE. The $^{68}GaCl_3$ solution was passed through the cationic cartridge, the eluate was scorned as it dragged the chemical and radionuclide contaminants from the eluate and gallium-68 was eluted with 800 µl acid acetone solution. The volume of this eluate was completed to 5 mL with 0.1M HCl.

This purified $^{68}GaCl_3$ solution was transferred to a labeling vessel containing 20 µg PSMA-HBED-CC and 1 mL of 1.5M sodium acetate buffer pH 6. The final solution was heated in block heater for 10 minutes at 90 ° C. The final solution of the radiolabeled product had pH 5. The range of activity employed in the radiolabeling was 222 to 370 MBq (6 to 10 mCi). The radiochemical purity of the labels was determined using the previously described Thin-film chromatography with ITLC-SG method.

In order to increase the percentage of radiochemical purity, was developed the filtration of the final radiolabeled product in a 0.22 µm filter membrane. The radiochemical purity of the labels was determined using the previously described Thin-film chromatography with ITLC-SG method.

2.5. Non-automated radiolabeling of PSMA-HBED-CC with $^{68}GaCl_3$ eluted from $^{68}Ge/^{68}Ga$ generator of manufacturer B

The radiochemical purity of the labeling was also studied using $^{68}GaCl_3$ solution eluted from $^{68}Ge/^{68}Ga$ generator from manufacturer B, GMP, without previous purification. The elution was performed with 5 mL of 0.05 M HCl, according to manufacturer's specification. The radiolabeling was performed under the same conditions described above, with the final product having pH 5. The range of activity employed in the radiolabeling was 148 to 222 MBq (4 to 6 mCi). No turbidity was observed. The radiochemical purity of the labels was determined using the previously described Thin-film chromatography with ITLC-SG method.

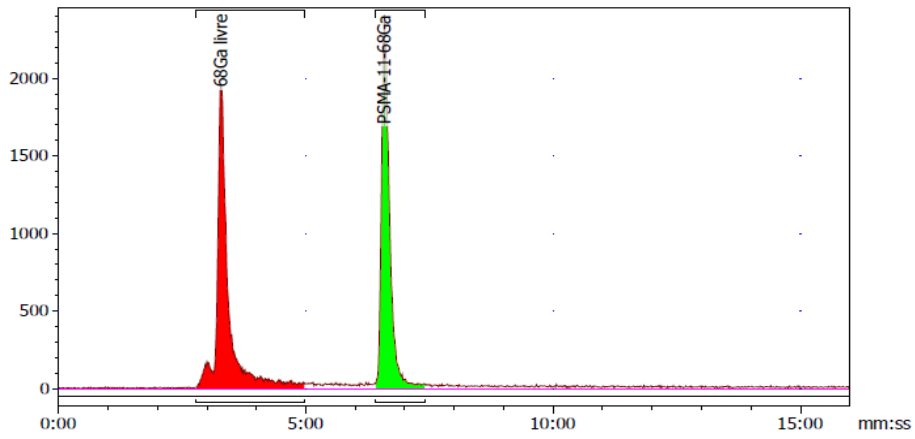
3. RESULTS AND DISCUSSION

The evaluation of the chromatographic systems used in the determination of the radiochemical purity in thin layer chromatography showed that the mobile phase methanol: ammonium acetate 1M (v/v) pH 8.5 retains the free $^{68}GaCl_3$ species at the origin of the tape, allowing the labeled product to migrate to the end of the chromatographic tape, facilitating the separation of the compounds. The same does not happen with the mobile phase citrate buffer: citric acid 1M (v/v) pH 5, whereas in this system the $^{68}GaCl_3$ migrates to the end of the chromatographic tape together with the labeled product. The two chromatographic supports of the thin layer chromatography presented the same profiles and the ITLC support was chosen for finalizing the chromatographic run in only 30 minutes.

The mean percentage of radiochemical purity (% PR) of 6 non-automated radiolabaling of PSMA-HBED-CC using generator eluent from de manufacturer A without prior purification, evaluated in thin layer chromatography, was 28,0 ± 7,4 %. The low radiolabeling yield is probably related to the presence of high levels of trivalent metals in the generator eluate, in addition to a percentage of ^{68}Ge above the limit specified in the European Pharmacopoeia.

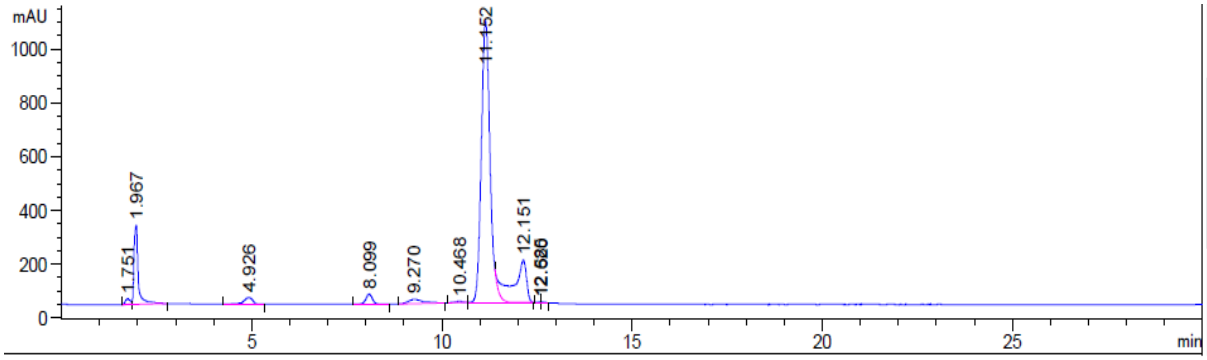
Figure 1 shows the chromatographic profile in CLAE of the radiolabeling mixture using $^{68}GaCl_3$ without prior purification.

Figure 1: *Profile of PSMA-HBED-CC labeled with $^{68}GaCl_3$ eluted from $^{68}Ge/^{68}Ga$ generator from manufacturer A, according to the previously described method.*



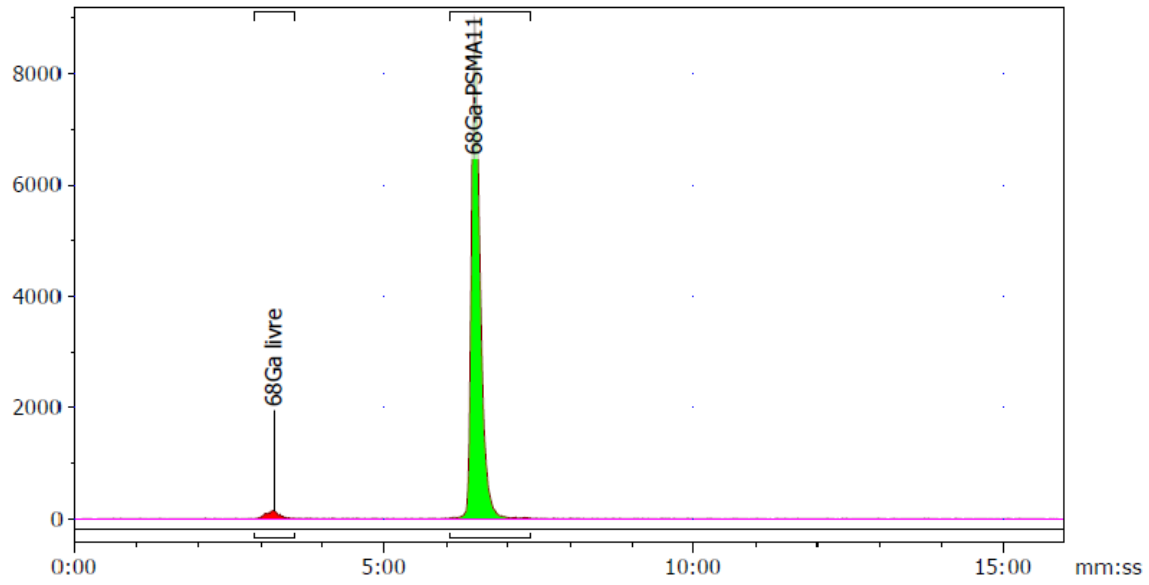
In the six non-automated PSMA-HBED-CC radiolabeling developed with $^{68}GaCl_3$ eluted from the manufacturer's generator A and previously purified on a cationic resin column, the mean percentage of radiochemical purity analyzed in thin layer chromatography ITLC-SG was 81.6 ± 7.4 %, showing a significant increase when compared to labeling without purification, but still below the percentage specified in the proposed European Pharmacopoeia monograph (> 91%).

Figure 2: *Profile of PSMA-HBED-CC labeled with $^{68}GaCl_3$ eluted from $^{68}Ge/^{68}Ga$ generator from manufacturer A, previously purified on a cationic resin column.*



The final solution of the labeled product also presented slight turbidity, which was removed after filtration of the radiolabeling mixture in the 0.22 µm filter membrane. The filtration promoted an increase in the mean radiochemical purity percentage to 92.5 ± 1.33 %. Thus, the use of non-GMP generator eluate only achieved satisfactory results of radiochemical purity when the eluate was previously purified on a cationic column to remove excess metal and ^{68}Ge contaminants and the final product was filtered on a 0.22 µm membrane, conditions that simulate automated synthesis module radiolabeling.

Figure 3: *Profile of PSMA-HBED-CC labeled with $^{68}GaCl_3$ eluted from $^{68}Ge/^{68}Ga$ generator from manufacturer A, previously purified on a cationic resin column and filtered on a 0.22 µm membrane.*



On the other hand, considering the non-automated radiolabeling of PSMA-HBED-CC with $^{68}GaCl_3$ eluted from the $^{68}Ge/^{68}Ga$ generator from manufacturer B, GMP, the results showed a percentage of radiochemical purity of 91 ± 1.52 % (N=2), according to the pharmacopoeial specification, without the need for prior purification of the eluate. In this case, the radiolabeling mixture appeared clear. The acid solution of gallium chloride eluted from the GMP generator meets the pharmacopoeial specifications for the limits of ^{68}Ge and metallic contaminants, allowing this use in direct radiolabeling procedure, using kits for ready radiolabeling and without the use of automated synthesis module, since labeling results in radiochemical purity that meets the pharmacopoeial criteria and without the need for further purification.

4. CONCLUSION

This study demonstrated that the non-automated labeling of PSMA-HBED-CC with ^{68}Ga, using a direct method that simulates the use of a kit for ready radiolabeling resulted in a satisfactory radiochemical purity percentage only when using $^{68}Ge/^{68}Ga$ grade GMP generator eluate demonstrating

the importance of the use of generator eluate that meets the pharmacopoeial purity criteria when it comes to non-automated direct labeling.

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