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*ORIGINAL RESEARCH ARTICLE*

# Development, formulation and quality evaluation of a lyophilized PSMA-11 kit for rapid radiolabeling with [$^{68}$Ga]Gallium

**Muhammad Fakhrurazi Ahmad Fadzil[1], Ooi Xin Yue[1], Zaitulhusna Md Safee[1], Nik Muhammad Fitri[1], Noratikah Mat Ail[1], Jaleezah Idris[2], Nor Idayu Razali[2], Hamdi Noor[2], Wan Hamirul Bahrin[3], Rahimah Rahim[3], Muhamad Faiz Othman[4], Zarif Ashhar[1]**

*[1]Pharmacy Department, Institut Kanser Negara, 4, Jalan P7, Presint 7, 62250 Wilayah Persekutuan Putrajaya, Malaysia*
*[2]Nuclear Medicine Department, Institut Kanser Negara, 4, Jalan P7, Presint 7, 62250 Wilayah Persekutuan Putrajaya, Malaysia*
*[3]Medical Technology Division, Malaysian Nuclear Agency, 43000 Kajang, Bangi, Malaysia*
*[4]Faculty of Pharmacy, MARA University of Technology (UiTM), Bandar Puncak Alam, Selangor, 42300 Puncak Alam, Malaysia*

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*Corresponding Author:
Muhammad Fakhrurazi Ahmad Fadzil
Address: Pharmacy Department, Institut Kanser Negara, 4, Jalan P7, Presint 7, 62250 Wilayah Persekutuan Putrajaya, Malaysia
Email: pfrazi@nci.gov.my

## ABSTRACT

**Introduction:** Recently, [$^{68}$Ga]Gallium lyophilized kit has been widely practiced as it simplifies the radiolabeling process. Thus, our aim is to develop, formulate and evaluate a single-vial lyophilized kit (EZYKit PSMA) for the preparation and radiolabeling of [$^{68}$Ga]Ga-PSMA-11.

**Methods:** Two commercially available [$^{68}$Ge/$^{68}$Ga] Generators used in this study were from ITG and Eckert & Ziegler with a current [$^{68}$Ga]Gallium elution activity of 222 MBq and 1480 MBq, respectively. Initially, EZYkit-PSMA radiolabeling parameters were optimized, and the Glass Transition Temperature (Tg) of the optimized PSMA-11 formulation was determined. Quality assessment of EZYkit-PSMA, including physical appearance, quantitative assay of the active ingredient, radiochemical purity, pH, sterility, endotoxin, and storage stability, were also established.

**Results:** Optimization of radiolabeling parameters showed that the highest radiochemical purity was achieved with 0.35 ml and 0.75 ml of 1.5 M acetate buffer and 10 µg PSMA-11 for both ITG and Eckert & Ziegler Generator, respectively. The quality assessment results of EZYkit-PSMA were within the specifications except for the physical appearance, where it appears to be structurally collapsed. This study discussed two main factors that contributed to the collapsed lyophilized cake.

**Conclusion:** Despite the physical appearance, EZYkit-PSMA was successfully developed, and the quality meets the pharmaceutical standards. EZYkit-PSMA showed high stability over 6 month's storage period in both the fridge and freezer. Overall, using EZYkit-PSMA for radiolabeling with [$^{68}$Ga]Gallium simplified the whole radiolabeling procedure and reduces process time and error with high labelling yield and efficiency.

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## INTRODUCTION

Prostate cancer is one of the most common types of cancer in men. According to Malaysian National Cancer Registry Report, prostate cancer is among the top ten most common cancers in elderly men [1]. The diagnosis of prostate cancer is usually performed by physical examination, biochemical markers, prostate-specific antigen (PSA) test, and conventional imaging techniques such as Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scan that detect morphological changes in the tissue [2]. Recently, the diagnosis of prostate cancer is entering a new level, utilizing molecular imaging techniques through Positron Emission Tomography-Computed Tomography (PET-CT). Unlike conventional imaging techniques, molecular imaging using PET-CT offers higher sensitivity and specificity [3]. In managing prostate cancer, imaging plays an important role in detecting primary tumor, initial staging, and therapy response evaluation [4]. The basis of molecular imaging techniques is by targeting a specific antigen that is overexpressed on prostate cancer cells known as the prostate specific membrane antigen (PSMA). PSMA is a type II integral membrane glycoprotein that was first detected in the human prostatic carcinoma cell line (LNCaP). PSMA was identified as a homologue of the protein N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALADase I or folate hydrolase I). Due to its selective overexpression in 90-100% of local prostate cancer lesions, as well as in cancerous lymph nodes and bone metastases, PSMA is a reliable tissue marker for prostate cancer and is considered an ideal target for molecular imaging technique [2, 4-6].

The utilization of small molecule PSMA inhibitors that consist of amino acids building block of Glutamate-Urea-Lysine is a promising molecule to target against the PSMA. The small molecule PSMA inhibitors is being radiolabel with various metal radioisotopes such as [$^{68}$Ga]Gallium, [$^{177}$Lu]Lutetium, [$^{90}$Y]Yttrium and [$^{225}$Ac]Actinium for both diagnosis and therapy of prostate cancer. At present, the gold standard small molecule PSMA inhibitors are PSMA-11 and PSMA-617 which uses two different types of bifunctional chelators; N,N-bis-[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N-diacetic acid (HBED) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), respectively. Currently, US FDA have approved two PSMA-based radioligand for diagnosis purposes that are PSMA-11 and PSMA-DCFPyl to be radiolabel with [$^{68}$Ga]Gallium and [$^{18}$F]Fluorine, respectively [6, 7] and one PSMA-based radioligand that is PSMA-617 to be radiolabel with [$^{177}$Lu]Lutetium for therapeutic purposes [8]. The radiolabeling of these radiometal isotopes with PSMA-11 and PMSA-617 follows a simple coordination complex which favors the formation of stable complex with the bifunctional chelators such as HBED and DOTA [9]. The [$^{68}$Ge/$^{68}$Ga] radionuclide generator provides an excellent source of positron-emitting [$^{68}$Ga]Gallium isotopes without needing an on-site cyclotron. [$^{68}$Ga]Gallium, with a half-life of 67.6 minutes, 89% positron branching ratio, and 8.9mm positron range, is an ideal PET emitter for diagnosis purposes [10]. It forms a more stable complex with acyclic chelators HBED (log $K = 38.5$) compared to macrocyclic DOTA (log $K= 21.3$) [11]. The complexation of [$^{68}$Ga]Gallium with the acyclic HBED is highly pH and temperature-dependent. In acidic condition, [$^{68}$Ga]Gallium exist as a free stable [$^{68}$Ga$^{3+}$] ion [12]. At a higher pH, it is hydrolyzed to form insoluble trihydroxide. Hence, to avoid this colloidal formation, a suitable buffer with weak metal complexing properties shall be incorporated [12, 13]. The optimal condition for [$^{68}$Ga]Gallium complexation with HBED is achieved in the pH range of 4.0-4.5 and a mild temperature of 50ºC [3, 14] . In our current setting, the preparation of [$^{68}$Ga]Ga-PSMA-11 involves multiple steps starting with the fractionation of PSMA-11 and followed by aliquoting the fractionated PSMA-11 with HEPES buffer for radiolabeling reaction. The use of HEPES buffer has been controversial as it is not described in any pharmacopeia and is not recognized as a substance for pharmaceutical use [13].

Recently, the use of lyophilized kit for radiolabeling [$^{68}$Ga]Gallium radioligand has been practiced elsewhere. [$^{68}$Ga]Gallium lyophilized kit follows the same strategies as the [$^{99m}$Tc]Technetium-based cold kits, which were developed as a ready-to-use single vial kit that eases the preparation for this short half-life radiopharmaceuticals. It is reported that the use of [$^{68}$Ga]Gallium lyophilized kit proves to be cost-effective, time-saving, and reduces human error during the fractionation and aliquoting the sample [11]. Thus, our aim is to develop, formulate and evaluate a single-vial lyophilized kit (EZYkit-PSMA) for the preparation and radiolabeling of [$^{68}$Ga]Ga-PSMA-11 for routine clinical use.

## METHODS

GMP Grade PSMA-11 was purchased from ABX advanced biochemical compounds (GmbH, Germany), Ultrapur 30% hydrochloric acid was purchased from Merck (Germany), sodium acetate anhydrous was purchased from Sigma Aldrich, and water for injection was purchased from Ain Medicare Sdn Bhd. The instruments used in this study were, calibrated dose calibrator from Biodex, miniGITA Dual thin layer chromatography was from Elysia Raytest Germany, High-Performance Liquid Chromatography (Agilent 1260 Infinity II) was from Agilent and calibrated pH meter used in this study was from Mettler Toledo. Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) was purchased from Agilent Technologies, and the SCHOTT lyophilization vial was from Adelphi Healthcare Packaging.

### *[$^{68}$Ge/$^{68}$Ga] generator*

Two commercially available [$^{68}$Ge/$^{68}$Ga] Generator used in this study were from ITG Germany (9 months old) and Eckert & Ziegler (1-2 months old) with a current elution activity of 222 MBq and 1480 MBq, respectively. The generator differs in the type of column materials where ITG utilizes organic material ($SiO_2$) while Eckert & Ziegler use titanium dioxide ($TiO_2$) as column resin. Each generator has an inlet and outlet polyethylene tube with luer fittings. [$^{68}$Ga]Gallium was eluted from the generator using 4.0 ml 0.05M hydrochloric acid and 5.0 ml 0.1 M hydrochloric acid for $SiO_2$- and $TiO_2$-based column generators, respectively. To limit metallic impurities such as $^{68}$Zn(II) in the [$^{68}$Ga]Gallium eluate, elution was performed 24 hours prior to radiolabelling. All [$^{68}$Ga]Gallium activity eluted from both generators was measured using a calibrated Biodex Dose Calibrator.

### *Optimization of radiolabeling parameters*

The radiolabeling of [$^{68}$Ga]Ga-PSMA-11 is highly pH, temperature, and time-dependent [9, 12]. These parameters were investigated with a constant radiolabeling temperature and time of 90°C and 10 minutes, respectively. The different volumes of 1.5 M sodium acetate buffer at pH 4.8 were tested with both types of [$^{68}$Ga]Gallium eluate from both $SiO_2$-based (ITG) and $TiO_2$-based (Eckert & Ziegler) [$^{68}$Ge/$^{68}$Ga] generator as detail out in Table 1. In addition, two different amounts of PSMA-11 were also investigated. The radiolabeling efficiency and pH for all samples were determined by radio instant thin layer chromatography silica gel (ITLC-SG) using 1.0 M ammonium acetate: methanol (1:1) as mobile phase and a calibrated pH meter (Mettler Toledo).

**Table 1.** Optimization of acetate buffer and PSMA-HBED -CC

| | Hydrochloric acid volume (ml) and molarity (M) | 1.5 M acetate buffer volume (ml) | Amount of PSMA-HBED-CC (nmol) |
|---|---|---|---|
| [$^{68}$Ga]Gallium eluate from ITG generator | 4.0 ml 0.05M | 0.25-0.40 | 5 and 10 |
| [$^{68}$Ga]Gallium eluate from Eckert & Ziegler generator | 5.0 ml 0.1M | 0.55-0.85 | 5 and 10 |

### *Lyophilization of PSMA-HBED-CC formulation (EZYkit-PSMA)*

The lyophilization process was performed in the Malaysian Nuclear Agency's cold kit manufacturing cleanroom. Prior to lyophilization, the Glass Transition Temperature (Tg) of the optimized PSMA-HBED formulation was determined using differential scanning calorimetry (DSC). Then, a stock solution containing 1000 nmol of PSMA-HBED-CC and 4.22 g sodium acetate was prepared aseptically in the final volume of 30 ml. The stock solution was mixed thoroughly, and aliquots of 0.3 ml were dispensed in a sterile SCHOTT lyophilization vial under Grade A laminar flow bench. The final formulation of the lyophilized kit is described in Table 2. After dispensing, the vials were immediately placed in a freeze-dryer (Labconco). The lyophilization process was carried out for 3 consecutive days under vacuum. A thermocouple was placed in one of the formulation vials to monitor the temperature during the lyophilization process.

**Table 2.** Final formulation of EZYkit-PSMA

| | Stock solution | Quantity / Vial |
|---|---|---|
| Sodium acetate (mg) | 4,220 | 42.2 |
| PSMA-HBED-CC (μg) | 1000 | 10 |
| Total volume (ml) | 30 | 0.3 |

### *Preliminary assessment of lyophilized PSMA-HBED-CC (EZYkit-PSMA)*

Lyophilized EZYkit-PSMA vials were radiolabeled with [$^{68}$Ga]Gallium eluate from ITG and Eckert & Ziegler [$^{68}$Ge/$^{68}$Ga] generator. The radiolabeling takes place via two different approaches depending on the types of [$^{68}$Ga]Gallium column. For ITG [$^{68}$Ge/$^{68}$Ga] generator, the [$^{68}$Ga]Gallium eluate was directly added into the lyophilized EZYkit-PSMA, and the mixture was heated at 90°C for 10 minutes. Meanwhile, for Eckert & Ziegler [$^{68}$Ge/$^{68}$Ga] generator, 0.4 ml formulation buffer containing 1.5M sodium acetate was added prior to generator elution. Then the [$^{68}$Ga]Gallium eluate was added into the vial and heated at 90°C for 10 minutes. The radiolabeling efficiency for all samples was determined as previously described.

### *Quality assessment of lyophilized PSMA-11 (EZYkit-PSMA)*

Quality assessment of the EZYkit-PSMA were conducted such as physical appearance (white dry powder/cake appearance), quantitative assay of active ingredient (±10%), radiolabeling pH (4-4.3), radiochemical purity (>95%), storage stability at two different storage temperature (2-8°C and -20°C), endotoxin (0.25 EU/ml) and sterility test.

The identity of the lyophilized PSMA-HBED-CC was examined using reversed phase High Performance Liquid Chromatography (HPLC) (Agilent Infinity II, USA). The method used C18 column (Luna, 3 x 150 mm, 3µm particle size, from Phenomenex, USA) at a flow rate of 0.6 ml/min. The detection of Ga-PSMA-11 standard reference and radiolabeled [$^{68}$Ga]Ga-PSMA-11 was performed using Agilent Infinity II VWD and Gabi Nova, Elysia Ray detector, respectively. The mobile phase A (0.1% trifluoroacetic acid (TFA) in water) and mobile phase B (0.1% TFA in acetonitrile). The gradient elution was shown in Table 3.

**Table 3.** Gradient elution condition in HPLC analysis of the lyophilized PSMA-HBED-CC

| Time | Mobile phase A | Mobile phase B |
|---|---|---|
| **0-0.5 min** | 95% | 5% |
| **0.5-10 min** | 95-60% | 5-40% |
| **10-12 min** | 60% | 40% |

### *Physical appearance*

The physical appearance of the EZYkit-PSMA was determined through visual observation. The lyophilized kit should appear in dry powder, or cake-like appearance with no moisture detected visually.

### *Quantitative assay of active ingredient*

Quantitative assay of the active ingredient PSMA-11 in the EZYkit-PSMA was determined using Agilent 1260 Infinity II High-Performance Liquid Chromatography. The calibration curves were constructed by plotting the peak area against four concentrations of PSMA-HBED-CC standards; 2.5µg/ml, 5µg/ml, 15µg/ml, and 30µg/ml. The correlation coefficient values ($R^2$) will be determined based on the linear equation. The content of PSMA-HBED-CC in the lyophilized kit should be within 10 µg ± 10%.

### *Radiolabeling pH and radiochemical Purity*

The radiolabeling was conducted, and the pH and radiochemical purity were assessed as previously described. The radiolabeling pH and radiochemical purity shall be within 4-4.3 and equal to or more than 95%, respectively.

### *Endotoxin and sterility*

Samples for endotoxin and sterility test were tested by Medical Technology Division, Malaysian Nuclear Agency. Initially, three vials out of 29 vials produced were sent for sterility and endotoxin test to ensure the whole aseptic filling and lyophilization process remains intact. The sterility and endotoxin tests were performed via the Direct Inoculation and Gel Clot methods.

### *Storage stability*

The storage stability for EZYkit-PSMA was evaluated at two different storage conditions: 2-8°C and -20°C for six months. Each month, the radiolabeling efficiency was determined for the lyophilized kit in different storage conditions. The radiochemical purity (%RCP) was determined through the radio ITLC method as previously described. Besides that, the endotoxin and sterility were tested again after 6 months for both storage conditions, as described previously.

## RESULTS

### *Optimization of radiolabeling parameters*

The different volumes of acetate buffer and PSMA-HBED-CC quantity were optimized to formulate the lyophilized kit. Figure 1 shows the %RCP of [$^{68}$Ga]Gallium eluate in 4.1 ml of 0.05 M HCl with different volumes of 1.5 M acetate buffer. The highest radiochemical purity (99.8%) was achieved with 0.35 ml of acetate buffer and 10 µg PSMA-11 with a fixed radiolabeling temperature of 90°C for 10 minutes. The pH of the mixture was 4.11. When using 5 µg PSMA-11, under the same condition as previous, the highest RCP was 95.7%. Figure 2 shows the %RCP of [$^{68}$Ga]Gallium eluate in 5 ml of 0.1M HCl with different volumes range of 1.5M acetate buffer. The highest RCP (99.6%) was obtained in 10 µg of PSMA-HBED-CC (specific activity = 0.148GBq/nmol) with 0.75 ml of acetate buffer. The pH of the mixture is 4.15. Meanwhile, the highest RCP achieved with 5 µg PSMA-11 is 96.4%.

### *Lyophilization of EZYkit-PSMA*

The DSC data shows that Tg starts at -8.80°C with a midpoint at -1.36°C. DSC plays an important role in determining the formulation's physicochemical properties and establishing the critical formulation temperature data. The lyophilization cycle data are shown in Table 4, where the samples were first frozen down to -50°C for 7 hours. The temperature for primary drying was at

0°C, and the temperature increased to 25°C for secondary drying until complete full lyophilization cycle. A total number of 29 EZYkit-PSMA vials were produced after one PSMA-11 vial was used as an indicator for the temperature probe during the lyophilization cycle.

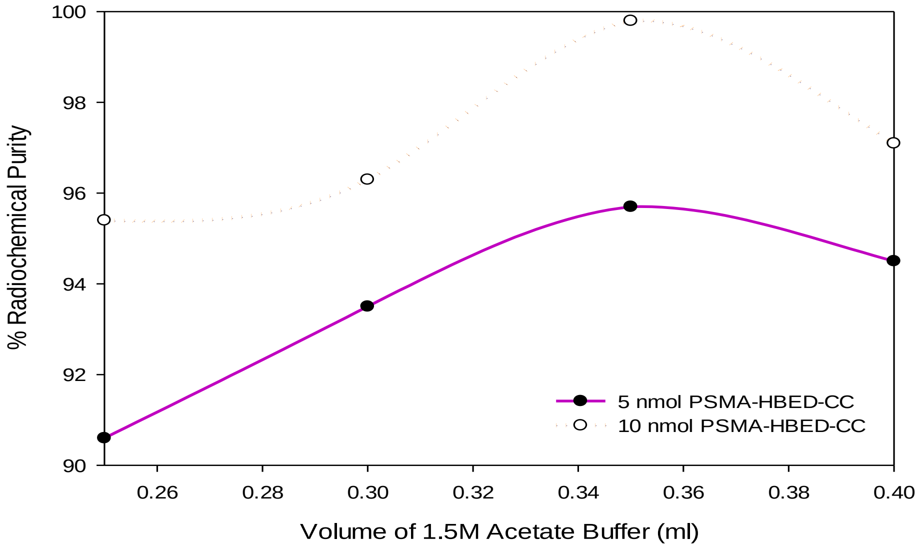

**Fig 1.** Radiochemical purity of [$^{68}$Ga]Gallium-PSMA-11 using ITG generator

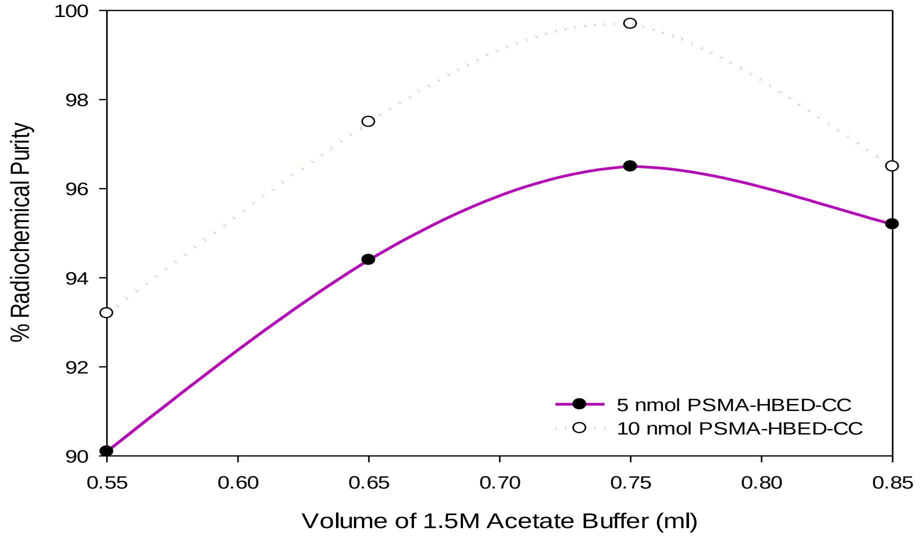

**Fig 2.** Radiochemical purity of [$^{68}$Ga]Gallium-PSMA-11 using Eckert & Ziegler generator

**Table 4.** Lyophilization cycle

| Process steps | Shelf temperature (°C) | Chamber vacuum (mTorr) | Duration (h) |
|---|---|---|---|
| Freezing | -50 | 0 | 7 |
| Primary drying | 0 | 60 | 25 |
| Secondary drying | 25 | 60 | 10-24 |

### *Preliminary assessment of EZYkit-PSMA*

Out of the 29 vials, four vials were immediately tested for radiolabeling using ITG and Eckert & Ziegler [$^{68}$Ga]Gallium generator, and the labeling pH was determined. Meanwhile, three (3) vials were immediately sent for sterility and endotoxin test. All results are within the specification as indicated in Table 5.

**Table 5.** Preliminary assessment of EZYkit-PSMA

| | **ITG Generator** | **Eckert & Ziegler Generator** |
|---|---|---|
| [$^{68}$Ga] Activity | 222 MBq | 1480 MBq |
| Radiochemical purity (n = 4) | 98.5 ± 0.4% | 96.1± 0.8% |
| pH (n = 4) | 4.2 ± 0.01 | 4.05 ± 0.13 |
| Sterility (n = 3) | Pass | Pass |
| Endotoxin (n = 3) | Pass | Pass |
| Total preparation time (n = 4) | 23 ± 1 minutes | 25 ± 1.5 minutes |

## *Quality assessment of EZYkit-PSMA*

Quality assessment results are summarized in Table 6. All parameters are within the specification except for physical appearance where the lyophilized structure of EZYkit-PSMA appeared to be in complete collapsed. The quantitative assay for PSMA-11 in EZYkit-PSMA was found to be 9.91 µg ± 0.8.

The identity for radiolabeled EZYkit-PSMA was presented in Figure 3, showing that the retention time for the radiolabeled [$^{68}$Ga]Ga-PSMA-11 was similar to the Ga-PSMA-11 standard reference.

**Table 6.** Quality assessment data for EZYkit-PSMA

| **No** | **Quality parameter** | **Acceptance criteria [reference]** | **Results** |
|---|---|---|---|
| 1 | Physical appearance (n = 29) | Dry powder or cake-like appearance [15] | Collapsed structure |
| 2 | Quantitative assay of active ingredient (n = 3) | 10 µg ± 10% of PSMA-HBED-CC (In-house optimization) | 9.91 µg ± 0.8 |
| 3 | Radiolabeling pH (n = 4) | 4 - 4.3 (In-house optimization) | 4.2 ± 0.01 |
| 4 | Radiochemical purity (n =4) | ≥ 95% (Ph. Eur) | 98 ± 0.4% |
| 5 | Endotoxin (n =3) | < 17.5 IU/ml (Ph. Eur) | Comply |
| 6 | Sterility (n =3) | Sterile (Ph. Eur) | Pass |

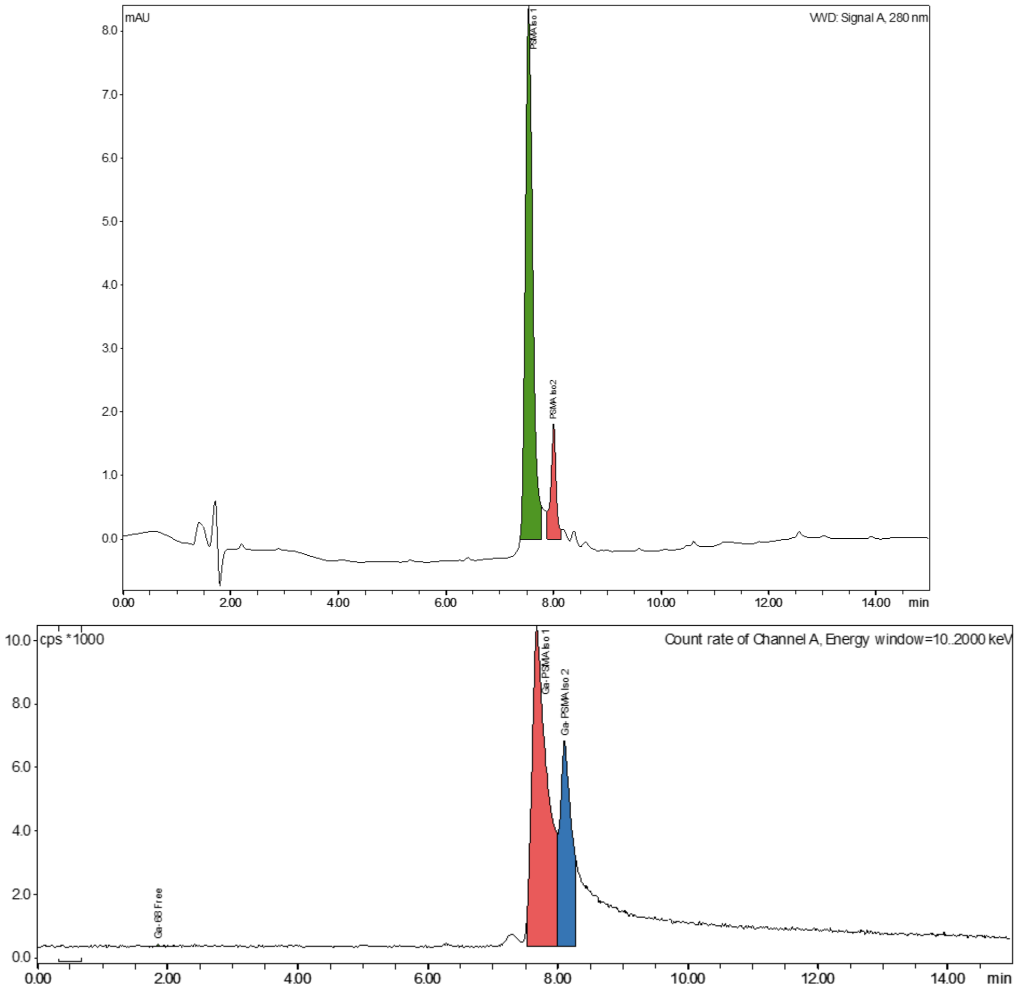

**Fig 3.** Identity test for radiolabeled [$^{68}$Ga]Ga-PSMA-11 using the lyophilized EZYkit-PSMA. UV chromatogram of Ga-PSMA standard reference (RT: 7.55 min stereoisomer 1, 8:00 min stereoisomer 2 (above); R-HPLC of radiolabeled [$^{68}$Ga]Ga-PSMA-11 (RT: 7.68 min stereoisomer 1, 8:12 min stereoisomer 2) (below)

### *Storage stability*

EZYkit-PSMA proves to be stable at both storage conditions of 2-8°C (fridge) and -20°C (freezer) for 6 months with the average radiochemical purity of 98.2 ± 0.5% and 97.1 ± 0.3%, respectively.

## DISCUSSION

In this study, the [$^{68}$Ga]Gallium eluted from both ITG and Eckert & Ziegler generator were used directly for radiolabeling with the PSMA-11 kit without purification and concentration of the eluate. Although some practices recommend to pretreated the [$^{68}$Ga]Gallium eluate using ion exchange cartridge method to eliminate metallic impurities and further concentrate the eluate volume to improve labeling yield [12, 14], our results proved that the PSMA-11 kit were able to radiolabel with untreated [$^{68}$Ga]Gallium Chloride ([$^{68}$Ga]$GaCl_3$). Comparing the amount of PSMA-11 used in this study, 10 µg was able to form higher complexation with [$^{68}$Ga]Gallium as compared to 5 µg of PSMA-11 for both types of [$^{68}$Ga]Gallium eluate from ITG and Eckert & Ziegler Generator. Theoretically, the higher the precursor amount, the more chelators will be available for [$^{68}$Ga]Gallium to be complexed, resulting in a higher radiolabeling yield [16]. The formulation for both ITG Generator and Eckert-Ziegler Generator was different since the molarity of the eluent solution was 0.05 M and 0.1 M, respectively. The radiolabelling pH was above 4 thus, the volume of 1.5 M acetate had to be higher for Eckert-Ziegler Generator. The selection of buffer was decided based on two important considerations: 1) pharmaceutically approved for humans; and 2) low complexation with [$^{68}$Ga]Gallium [13].

Lyophilization is a process that removes water component by sublimation that improves the stability of the pharmaceutical product for a longer period [17]. Generally, the lyophilization cycle comprises three stages that are 1) freezing the product (ice formation), 2) primary drying to remove ice (water) by sublimation, and 3) secondary drying to remove the remaining water, which is bound to the crystalline structure of the product [18]. During lyophilization process, it is critically important not to go beyond the Tg of the formulation mixture in the first stage of lyophilization cycle, which is the primary drying as it may lead to change in product morphology [19]. The interstitial space between the crystalline structure must be rigid enough so that during the secondary drying cycle, the water sublimation process does not cause the complete collapse of the lyophilized structure. We suspected that the collapse of the lyophilized structure was due to two main reasons: lyophilization parameter and excipients content. During the lyophilization cycle, we noticed that the primary drying temperature (0°C) was higher than the Tg temperature (-1.36°C at midpoint). Tg helps determine the maximum temperature the formulation can withstand during primary drying, and if the drying temperature goes above Tg temperature, a complete structural loss occurs [20]. The second reason is due to the lack of bulking agent such as mannitol or glycine during the lyophilization process. The bulking agent aids in maintaining a cake-like structure, especially during the primary and secondary drying cycle [17]. During the formulation of EZYkit-PSMA, we keep our formulation as simple as possible with only two main components: acetate buffer and the active pharmaceutical, which is the PSMA-HBED-CC; to avoid significant changes in the final lyophilized product that may affect overall quality [19]. Despite the physical appearance test not meeting the specifications, the quantitative assay of the active ingredient is within the specification of 10 µg ± 10%, proving that the lyophilization process does not cause significant loss of the active ingredient that may affect the radiolabeling yield.

Preliminary assessment of lyophilized EZYkit-PSMA showed that the radiolabeling with Eckert & Ziegler Generator is lower than the one with ITG Generator 96.1± 0.8% versus 98.5 ± 0.4% respectively (Table 5). The radiolabeling can be improved by pre-concentration of [$^{68}$Ga]Gallium eluate using fractionation or ion exchange resin [3]. However, in this study, we use the full [$^{68}$Ga]Gallium eluate volume from both generators as the idea is to develop a simplified and rapid radiolabeling procedure for the PSMA-11 kit. The total preparation time for both generators differs by 1-2 minutes as the Eckert & Ziegler generator requires an additional step of adding 400 µl of 1.5M acetate buffer before [$^{68}$Ga]Gallium eluate is reconstituted in the EZYkit-PSMA. The preparation time starts from generator elution or reconstitution of acetate buffer into the kit for Eckert & Ziegler generator until 10 minutes of cooling down of the sample after radiolabeling. Nevertheless, using the EZYkit-PSMA shorten the preparation time up to 5 minutes compared to using the traditional method with the fractionated PSMA that requires the preparation of buffer on-site.

Golan et al. presented improved radiochemical yield using their developed isoPROtrace-11 compared to automated synthesis [21]. The radiolabeling performed in their study was at room temperature for 5 minutes. The radiochemical purity obtained using the Eckert & Ziegler Generator was comparable to our work of 96.2 % ± 1.69 and 96.1% ± 0.8, respectively. Our work eliminated the manipulation required to concentrate the eluate to 2.5 ml which may risk variation in final activity and potentially the pH. However, the radiolabelling was performed at an elevated temperature for 10 minutes. The radiochemical purity was higher with ITG Generator since the 0.05 M HCl used minimizes the protonation of functional groups in ligands for chelating $^{68}$Ga radiopharmaceuticals [22].

The RCP was not much different in both storage conditions for a duration of 6 months. Thus, the lyophilized kit can be stored as a fridge item at 2-8°C without any problem. At the 6th months of storage interval, each EZYkit-PSMA vial was sent for sterility and endotoxin test to determine the integrity of the container closure system. Results showed that the product was deemed stable in long-term storage conditions.

## CONCLUSION

PSMA-11 lyophilized kit (EZYkit-PSMA) was successfully developed, and the quality meets the pharmaceutical standard except for physical appearance, which appears structurally collapsed. This was due to the primary drying temperature above Tg temperature and the absence of bulking agent, which may help to strengthen the amorphous structure during the water sublimation process. EZYkit-PSMA can be stored in both fridge and freezer as the kit showed high stability after 6 months. Overall, using the EZYkit-PSMA for radiolabeling with [$^{68}$Ga]Gallium for both ITG and Eckert & Ziegler generator simplified the whole radiolabeling procedure, reduce process time and error with high labeling yield and efficiency.

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