

# Preparation, quality control and biodistribution study of $^{68}\text{Ga}$ -BPAMD: Optimized production with an in-house $^{68}\text{Ge}$ - $^{68}\text{Ga}$ generator

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## ABSTRACT

**Introduction:** Bone metastases are common in the progression of various tumors leading to severe pain and decrease in quality of life. The aim of this study was to optimize the production of  $^{68}\text{Ga}$ -BPAMD as an ideal bone imaging agent using an in-house  $^{68}\text{Ge}/^{68}\text{Ga}$  generator for future clinical use.

**Methods:** The optimized conditions for the preparation of  $^{68}\text{Ga}$ -BPAMD were determined by varying ligand concentration, pH, time and temperature. The radiochemical purity of the complex was checked using ITLC method. The stability at room temperature and in human serum and the hydroxyapatite (HA) binding of the complex were studied. Biodistribution of  $^{68}\text{Ga}$ -BPAMD and  $^{68}\text{GaCl}_3$  were investigated in male Syrian rats.

**Results:** The radiolabelled compound was prepared with a radiochemical purity of >99% after 15 min at the optimized conditions (30  $\mu\text{g}$  of ligand, 90  $^\circ\text{C}$ , pH=3-5). The complex was stable in the final preparation and in the presence of human serum (>98%). HA binding assay demonstrated that at the amount of 10 and 25 mg of HA, 62.3 and 88.5 % of the complex are bound to HA, respectively. The agent demonstrated significant accumulation in the bone tissue, while cleared very fast from blood circulation. Major difference in uptake between  $^{68}\text{Ga}$ -BPAMD and  $^{68}\text{GaCl}_3$  was observed especially in blood, bone, liver, and spleen which can be considered as favorable characteristics of this agent.

**Conclusion:** According to these results, this agent can be produced with the recently developed in-house generator and considered as a worthy bone PET imaging agent available for further clinical use.

**Key words:** Bone metastasis; BPAMD;  $^{68}\text{Ga}$ ; PET scan

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## INTRODUCTION

Skeletal metastases are common in many cancers, most frequently in prostate, lung and breast malignancies and result in reduced survival, morbidity, pain and quality of life [1, 2]. With the significant associated morbidity, the introduction of new methods for early detection and response assessment of skeletal metastases has become even more important [3]. Imaging has an important role in the detection, diagnosis, prognostication, treatment planning, and follow-up monitoring of bone metastases and the overall treatment strategy [4].

Between the currently available imaging modalities, the two radionuclide functional techniques, scintigraphy and positron emission tomography (PET) imaging have major roles [5]. Skeletal scintigraphy with labelled phosphonates enables visualization of local bone metabolism, which is activated in an early phase of some types of cancer [4].  $^{99\text{m}}\text{Tc}$  labelled methylene diphosphonate ( $^{99\text{m}}\text{Tc}$ -MDP) is the most frequently used radiotracer for diagnostic purposes using SPECT [6].

Improvements in sensitivity and specificity diagnosis of the skeleton metastases with PET/CT tracers such as  $^{18}\text{F}$ -NaF or  $^{18}\text{F}$ -FDG have been reported in the recent years [3, 7].  $^{18}\text{F}$  as a cyclotron based radioisotope is less widely available requiring an on-site cyclotron due to its half-life. To date, the interesting physical properties and availability of gallium-68 as  $^{68}\text{Ge}/^{68}\text{Ga}$  commercial generator systems, make it an interesting nuclide for developing new PET tracers and it has the potential to become as useful for PET as  $^{99\text{m}}\text{Tc}$  has proved to be for SPECT imaging [8].

$^{68}\text{Ga}$  with a short physical half-life of 68 min decays 89% through positron emission (maximum energy of 1.92 MeV, mean 0.89 MeV). While its short physical half-life enables improved dosimetry and repeats imaging, the long physical  $t_{1/2}$  of the parent radionuclide (270.8 d) allows the use of the generator up to one year [9]. Recently, numerous  $^{68}\text{Ga}$ -labelled pharmaceuticals have been developed and employed in preclinical and limited clinical trials [9, 10].

With the introduction of a new macrocyclic diphosphonate, (4- $\{[(\text{bis}(\text{phosphonomethyl})\text{carbamoyl})\text{methyl}]-7,10\text{-bis}(\text{carboxymethyl})-1,4,7,10\text{-tetraazacyclododec-1-yl}\}$  acetic acid (BPAMD), overcoming some of the restrictions of EDTMP. Preparation of  $^{68}\text{Ga}$ -BPAMD has been reported demonstrating intense accumulation in osteoblastic lesions in the first human study in a patient with extensive bone metastases of prostate cancer [11].

In this research, with regard to the reported favorable characteristics of  $^{68}\text{Ga}$ -BPAMD for detection of bone metastases, the authors tried to prepare this radiolabelled compound and develop an optimized

method for the preparation using the in-house developed  $^{68}\text{Ge}/^{68}\text{Ga}$  generator. Besides, biodistribution of the complex in male Syrian rats was studied.

## METHODS

The  $^{68}\text{Ge}/^{68}\text{Ga}$  generator with the nominal activity of 30 mCi was obtained from Pars Isotope Co. (Tehran, Iran).

BPAMD was provided from ABX (Radeberg, Germany). All other chemical reagents were purchased from Merck (Darmstadt, Germany). Radionuclidic purity was checked using a high purity germanium (HPGe) detector coupled with a Canberra<sup>TM</sup> (model GC1020-7500SL) multichannel analyzer. A bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA) and Whatman No. 2 paper (Whatman, Buckinghamshire, U.K.) were used for the assessment of radiochemical purity. An ICP-OES spectrometer (Varian Co., model Turbo-AX-150-Liberty) was employed for chemical purity investigation. All rats were kept at routine day/night light program and under common rodent diet pellets. All values were expressed as the mean  $\pm$  standard deviation (Mean  $\pm$  SD) and the data were compared using student T-test. Statistical significance was defined as  $P < 0.05$ .

### Preparation and quality control of $^{68}\text{Ga}$ -BPAMD

Elution of  $^{68}\text{Ge}/^{68}\text{Ga}$  generator and quality control of  $^{68}\text{GaCl}_3$  solution was performed in accordance to the previously reported literature [12, 13]. In order to prepare  $^{68}\text{Ga}$ -BPAMD with the maximum complexation yields, several experiments were carried out and the effect of various parameters on the labeling yield were studied including ligand concentration, pH, temperature and reaction time.

A stock solution of BPAMD was prepared by dissolving in the distilled water with the concentration of 1 mg/mL. 20-50  $\mu\text{L}$  of the stock solution was added to the borosilicate vials containing a certain volume of  $^{68}\text{GaCl}_3$ . While pH of the reaction vials was adjusted to 3-5, the vials were putted in a hot water bath (with 50-90  $^{\circ}\text{C}$ ) for 5-30 minutes. Finally, the radiolabelled compound was passed over the strong cation exchanger (Strata-X-C 60 mg) preconditioned with 1 mL 4 M HCl and 1 mL water, respectively.

The radiochemical purity of the final compound was checked using ITLC method. For this purpose, various mobile phase mixtures (10% ammonium acetate: methanol (1:1), 0.25 M sodium citrate, ammonium hydroxide: methanol: water (0.2:2:4) and acetone: acetic acid (1:3)) were applied, whereas, whatman No.2 was employed as the stationary phase.

### Stability studies

The stability of the complex at room temperature (22°C) and in presence of freshly-prepared human serum (at 37°C) was studied at different time intervals by ITLC method. For this purpose, Whatman No.2 and  $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$  (2:20:40) were selected as stationary and mobile phases, respectively.

### Hydroxyapatite binding assay

HA binding assay was performed according to the previously reported research [14]. Briefly, 10 and 25.0 mg of solid HA was placed in reaction vials, while 2 ml of saline solution (pH 7.4) was added to each vial and the mixtures were shaken for 24 h. After adding 50  $\mu\text{l}$  of the radioactive preparation, shaking was continued for 15 min. The suspensions were centrifuged, and two aliquots of the supernatant liquid were taken from each vial and the radioactivity was measured with a well-type counter. Test experiments were performed using a similar procedure, but in the absence of HA. The percentage binding of  $^{68}\text{Ga}$  to HA was calculated according to  $\text{HB} = 1 - \text{A/B} \times 100$ , where A is the mean radioactivity value of the supernatant sample under study and B is the mean total value of whole activity used.

### Biodistribution assessment of $^{68}\text{Ga}$ -BPAMD in male Syrian rats

100  $\mu\text{L}$  of the final radiolabelled compound (with approximately 5.55 MBq radioactivity) was injected intravenously into the male Syrian rats through their tail veins. The rats weighting 180-220 g kept at routine day/night light program and under common rodent diet pellets, were sacrificed at the selected intervals (15, 30, 60 and 120 m) after injection (n=4). The tissues (kidney, liver, spleen, lung, stomach, pancreas, intestine, bone, heart, muscle, adrenal and skin) were weighed and rinsed with normal saline and their activities were determined by means of a p-type HPGe detector. The percentage of injected dose per gram (%ID/g) for each organ was calculated by dividing the activity of each organ at the specified time to the total injected activity and mass of each organ.

Values were expressed as mean  $\pm$  standard deviation and the data were compared using Student's T-test. The statistical significance was defined as  $P < 0.05$ .

## RESULTS

### Preparation and quality control of $^{68}\text{GaCl}_3$

The concentrations of tin, zinc and copper in the eluted  $^{68}\text{Ga}$  using ICP-OES were evaluated as <0.1, 0.23 and 0.38, respectively. While, gamma spectrometry showed the presence of 511 and 1077 keV, all originating from  $^{68}\text{Ga}$ , the radiochemical purity of  $^{68}\text{GaCl}_3$  sample was estimated to be more than 99.9%.

### Preparation and quality control of $^{68}\text{Ga}$ -BPAMD

In order to obtain maximum complexation yields, several experiments were carried out by varying pH, ligand concentration, time and temperature. The effect of ligand concentration on radiochemical purity was indicated in Figure 1.

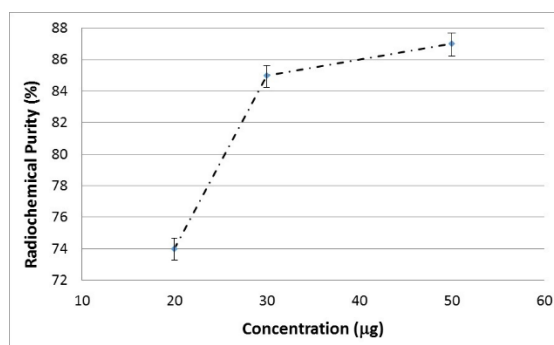


Fig 1. Radiochemical purity of  $^{68}\text{Ga}$ -BPAMD versus ligand concentration at pH=3, temperature of 90°C and time of 30 min.

The results showed increasing of radiochemical purity by the growth of concentration until the amount of BPAMD reaches 30  $\mu\text{g}$ , since then the radiochemical purity remains approximately constant.

The effect of pH on radiochemical purity was also investigated using HEPES (Figure 2).

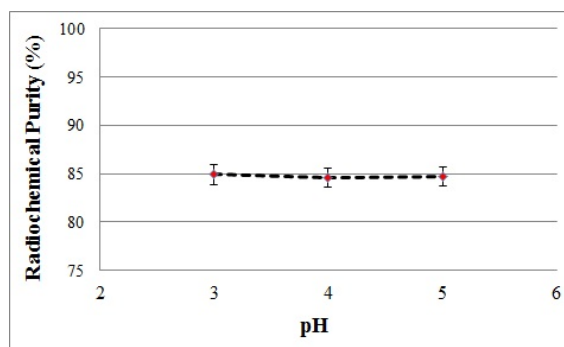
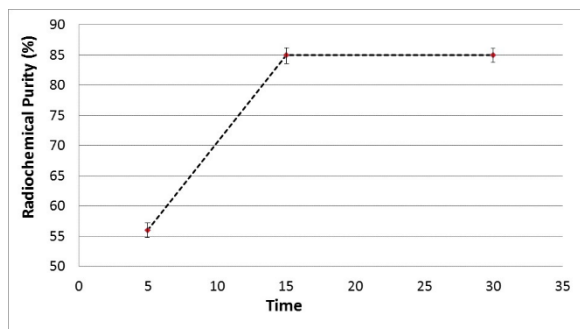


Fig 2. Radiochemical purity of  $^{68}\text{Ga}$ -BPAMD versus pH, at temperature of 90°C, time of 30 min and ligand concentration of 30  $\mu\text{g}$ .

The experiments indicated almost no change in radiochemical purity by alteration of pH from 3 to 5. As a result, pH of 3 to 5 can be considered as an appropriate choice.

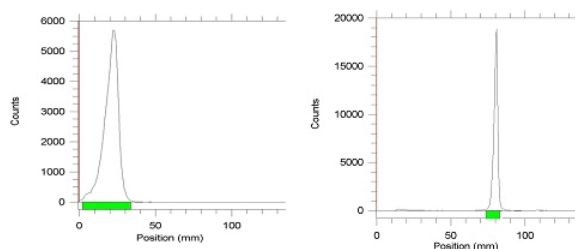
Radiochemical purity was checked at different intervals (Figure 3).

According to these data, radiochemical purity does not change after 15 min and this time is adequate for preparation of this radiolabelled compound.



**Fig 3.** Radiochemical purity of  $^{68}\text{Ga}$ -BPAMD versus time at pH=3, temperature of  $90^\circ\text{C}$  and ligand concentration of  $30\ \mu\text{g}$ .

Radiochemical purity of the radiolabelled complex was assessed by ITLC method using different chromatographic systems. Among these solvent systems, ammonium hydroxide: methanol: water (0.2:2:4) and acetone: acetic acid (1:3) were considered as the suitable mobile phase. Using these solvents and Whatman No. 2 paper, the free cation remains at the origin while the radiolabelled compound migrates to higher  $R_f$  (0.8) (Figure 4).



**Fig 4.** ITLC chromatogram of  $^{68}\text{GaCl}_3$  (right) and  $^{68}\text{Ga}$ -BPAMD (left) in  $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$  (2:20:40) using Whatman No. 2 as a stationary phase.

### Stability studies

Stability of  $^{68}\text{Ga}$ -BPAMD at room temperature was investigated up to 120 min after preparation. The radiochemical purity of the complex remained  $>98\%$ . Incubation of labelled complex in freshly prepared human serum for 120 min at  $37^\circ\text{C}$  showed no loss of  $^{68}\text{Ga}$  from the complex.

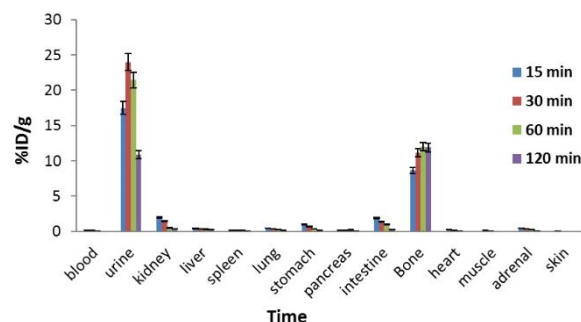
### Hydroxyapatite binding assay

HA assay demonstrated high capacity binding for  $^{68}\text{Ga}$ -BPAMD to HA. At the amount of 10 and 25 mg of HA, 62.3 and 88.5 % of the complex are bound to HA, respectively.

### Biodistribution assessment of $^{68}\text{Ga}$ -BPAMD in male Syrian rats

The percentage of injected dose per gram in rat organs was determined up to 120 m after injection of  $^{68}\text{Ga}$ -

BPAMD (Figure 5). As expected, most of the injected activity was accumulated into the bones. The results showed very fast blood clearance. The urinary tract is the major route of excretion for the labelled compound.



**Fig 5.** Percentage of injected dose per gram (%ID/g) in different rat organs after 15, 30, 60 and 120 m of  $^{68}\text{Ga}$ -BPAMD ( $5.55\ \text{MBq}$ ) injection.

## DISCUSSION

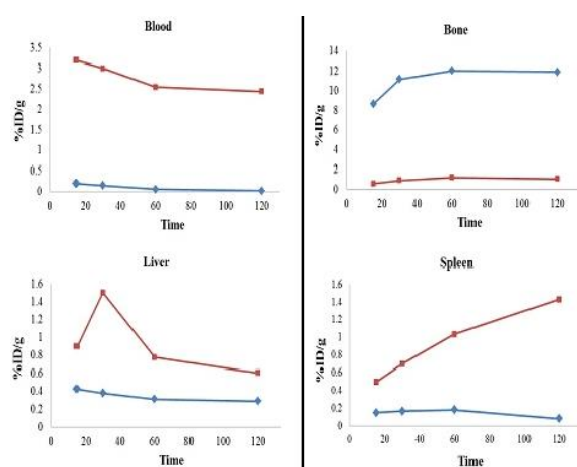
With the preferential accumulation of MDP in bones and its capability to label by  $^{99\text{m}}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ -MDP has been recognized as a standard radiopharmaceutical for scintigraphic imaging of bone lesions [15]. The slow clearance of  $^{99\text{m}}\text{Tc}$ -MDP from the soft tissues, its radiation burden and low specificity have the researchers to look for other novel agents overcoming these shortcomings.

While BPAMD as a novel macrocyclic diphosphonate with better characteristics compared with the first generation phosphonates, some diagnostic and therapeutic radiolabelled complexes of this new agent have been reported. Favorable properties of  $^{68}\text{Ga}$ ,  $^{68}\text{Ga}$ -BPAMD namely high target to soft tissue ratios and fast renal clearance is reported in the first human study [16].

Regarding these promising results, we in this study produced  $^{68}\text{Ga}$ -BPAMD was for the first time in the country using the new generator developed by Pars Isotope Company. We were successful in production of this new bone imaging agent with radiochemical purity of higher than 99% at the optimized conditions. Biodistribution of  $^{68}\text{Ga}$ -BPAMD in male Syrian rats was studied indicating high accumulation in the bone and fast blood clearance. Also for better characterization and comparison, biodistribution of  $^{68}\text{Ga}$  was assessed. The percentage of injected dose per gram following administration of  $^{68}\text{Ga}$ -BPAMD and  $^{68}\text{GaCl}_3$  in blood and other vital organs are indicated in Figure 6.

As shown, the radiolabelled complex cleared very fast from blood circulation with almost complete clearance of activity from blood after 120 min, whereas, the activity of  $^{68}\text{GaCl}_3$  in blood is considerably higher and

shows the slower clearance. As expected, the data demonstrated the greater accumulation of  $^{68}\text{Ga}$ -BPAMD in bone.



**Fig 6.** Comparative organ uptake of  $^{68}\text{Ga}$ -BPAMD (blue line) and  $^{68}\text{GaCl}_3$  (red line) in Syrian rats.

Accumulation of the complex in the intestine is higher until 60 min, however, lesser aggregation of the activity in liver and spleen can be considered as an ideal characteristic of this radiolabelled complex.

### CONCLUSION

In this study,  $^{68}\text{Ga}$ -BPAMD was prepared successfully with the generator recently developed in Iran. The radiochemical purity of higher than 99% after 15 min of preparation was achieved at the optimized conditions (30  $\mu\text{g}$  of ligand, 90  $^\circ\text{C}$ ,  $\text{pH}=3-5$ ). The stability of the radiolabelled complex at room temperature and in human serum at 37  $^\circ\text{C}$  showed almost no decrease in the radiochemical purity even after 120 min. The biological behavior of this new complex was investigated after intravenous injection into the male Syrian rats indicated significant accumulation in bone. The data was compared with the biodistribution of  $^{68}\text{GaCl}_3$  in the same-type rats demonstrating a major difference in uptake, especially in blood, bone, liver, and spleen. According to these results,  $^{68}\text{Ga}$ -BPAMD can be produced with the recently developed in-house generator and be considered as a worthy bone PET imaging agent in the country.

### REFERENCES

1. Singh VA, Haseeb A, Alkubaisi AA. Incidence and outcome of bone metastatic disease at University Malaya Medical Centre. *Singapore Med J.* 2014 Oct;55(10):539-46.
2. Kuchuk I, Hutton B, Moretto P, Ng T, Addison CL, Clemons M. Incidence, consequences and treatment of bone metastases in breast cancer patients-Experience from

a single cancer centre. *J Bone Oncol.* 2013 Oct 3;2(4):137-44.

3. Cook GJ, Azad GK, Goh V. Imaging bone metastases in breast cancer: Staging and response assessment. *J Nucl Med.* 2016 Feb;57 Suppl 1:27S-33S.
4. Heindel W, Gübitz R, Vieth V, Weckesser M, Schober O, Schäfers M. The diagnostic imaging of bone metastases. *Dtsch Arztebl Int.* 2014 Oct 31;111(44):741-7.
5. Cuccurullo V1, Cascini GL, Tamburrini O, Rotondo A, Mansi L. Bone metastases radiopharmaceuticals: an overview. *Curr Radiopharm.* 2013 Mar;6(1):41-7.
6. Nilegaonkar S, Sonar S, Ranade A, Khadilkar M. TC99m MDP bone scan in evaluation of painful scoliosis. *Indian J Nucl Med.* 2010;25(2):67-69.
7. Araz M, Aras G, Küçük ÖN. The role of 18F-NaF PET/CT in metastatic bone disease. *J Bone Oncol.* 2015 Sep 16;4(3):92-7.
8. Banerjee SR, Pomper MG. Clinical applications of Gallium-68. *Appl Radiat Isot.* 2013 Jun;76:2-13.
9. Jalilian AR. An overview on Ga-68 radiopharmaceuticals for positron emission tomography applications. *Iran J Nucl Med.* 2016;24(1):1-10.
10. Naderi M, Zolghadri S, Yousefnia H, Ramazani A, Jalilian AR. Preclinical study of  $^{68}\text{Ga}$ -DOTATOC: Biodistribution assessment in syrian rats and evaluation of absorbed dose in human organs. *Asia Ocean J Nucl Med Biol.* 2016 Winter;4(1):19-29.
11. Fellner M, Baum RP, Kubíček V, Hermann P, Lukes I, Prasad V, Rösch F. PET/CT imaging of osteoblastic bone metastases with ( $^{68}\text{Ga}$ )-bisphosphonates: first human study. *Eur J Nucl Med Mol Imaging.* 2010 Apr;37(4):834.
12. Mirzaei A, Jalilian AR, Aghanejad A, Mazidi M, Yousefnia H, Shabani G, Ardaneh K, Geramifar P, Beiki D. Preparation and Evaluation of ( $^{68}\text{Ga}$ )-ECC as a PET Renal Imaging Agent. *Nucl Med Mol Imaging.* 2015 Sep;49(3):208-16.
13. Aghanejad A, Jalilian AR, Maus S, Yousefnia H, Geramifar P, Beiki D. Optimized production and quality control of  $^{68}\text{Ga}$ -Dotatate. *Iran J Nucl Med.* 2016;24(1):29-36.
14. Yousefnia H, Zolghadri S, Jalilian AR. Preparation and biodistribution assessment of  $^{111}\text{In}$ -BPAMD as a novel agent for bone SPECT imaging. *Radiochim Acta.* 2015;103(9):653-661.
15. Doroudi A, Saadati SM, Ahmadi F, khodayar J, Rezaee S, Heidari Kaydan H. The effect of prednisolone on the sensitivity of Technetium 99m-methylene diphosphonate ( $^{99\text{m}}\text{Tc}$ -MDP) scintigraphy to detect simulated closed fracture in the rat tibia. *Iran J Nucl Med* 2012;20(1):32-38.
16. Fellner M, Baum RP, Kubíček V, Hermann P, Lukes I, Prasad V, Rösch F. PET/CT imaging of osteoblastic bone metastases with ( $^{68}\text{Ga}$ )-bisphosphonates: first human study. *Eur J Nucl Med Mol Imaging.* 2010 Apr;37(4):834.