RPE | Radiation Physics & Engineering
A Peer-Reviewed Journal
Published by K.N. Toosi University of Technology
http://rpe.kntu.ac.ir
Research Article

Radiation Physics and Engineering 2025; 6(2):41–46

# Comparison of radiolabeled bisphosphonates in bone metastasis

Mostafa Erfani*, Azadeh Mikaeili, Mostafa Goudarzi, Zhila Fallah, Maryam Rezaei

*Radiation Application Research School, Nuclear Science and Technology Research Institute (NSTRI), Tehran, Iran*

## HIGHLIGHTS

- new generations of bisphosphonate tracers have been developed.
- Prepared conjugate showed high labeling yields and specific activity.
- The tracers were stable in saline solution.
- High bone uptake followed by low liver uptake are obtained at early time point and for third generation bisphosphonate.

## ABSTRACT

Bisphosphonates have a very high affinity for bone minerals because they bind to hydroxyapatite crystals. Based on this fact, it was hypothesized to evaluate bone uptake for $^{99m}$Tc/$^{188}$Re radiolabeled bisphosphonates to determine the order of uptake in the bone as agents for imaging or treatment of skeletal disorders. Radiolabeling of bisphosphonate samples were performed by adding 185 MBq of $^{99m}$Tc/$^{188}$Re in saline, and $SnCl_2$, $2H_2O$ and ascorbic acid as reducing agents. Radioactive thin layer chromatography (RTLC) was used to determine the radiochemical purity. The stability of radiocomplexes was investigated in saline and human serum. Bone uptake was evaluated through biodistribution experiments in normal mice. A radiochemical purity of $> 90\%$ was obtained for evaluated radiotracers. Bone accumulation and rapid renal excretion were observed in the biological assessment of all evaluated bisphosphonates at 2 hours post injection. Although bone uptake was desirable for investigated bisphosphonates, it appears that third-generation bisphosphonates can be used as novel bone imaging or therapeutic agents.

## KEYWORDS

$^{99m}$Tc/$^{188}$Re
Radiochemical yield
Biodistribution
Imaging

## HISTORY

Received: 10 July 2024
Revised: 12 November 2024
Accepted: 15 November 2024
Published: Spring 2025

## 1 Introduction

The use of bisphosphonates for various bone problems has been on the rise. Because bisphosphonates are bonded to hydroxyapatite crystals, they have a very high affinity for bone minerals (Russell, 2006). As a result, the availability of hydroxyapatite binding sites determines the retention of skeletal bisphosphonates. When skeletal turnover accelerates, bisphosphonates are preferentially absorbed into sites of active bone remodeling (Fleisch et al., 1966). Bisphosphonates that are not retained in the skeleton are rapidly removed from the blood stream by renal excretion. Bisphosphonates prevent calcification and break down of hydroxyapatite, which effectively stops bone resorption (Russell et al., 1970). According to recent research, bisphosphonates may also prevent osteoblast and osteocyte apoptosis.

The physical properties of technetium-99m and its availability, as it can be produced using a generator, make it easy to use. It has therefore become the most important radionuclide for organ imaging. Numerous $^{99m}$Tc-labeled phosphate compounds have been produced for skeletal imaging, as bisphosphonates have been reported to have a strong affinity for bone minerals (King et al., 1980; Valdez and Jacobstein, 1980). Pyrophosphate is a basic polyphosphate with just two phosphate moieties. Today, [$^{99m}$Tc]Tc-pyrophosphate is no longer used for bone scanning because of soft tissue uptake, but it is still useful for detecting myocardial infarction. Unfortunately, phosphating agents can be affected or damaged in vivo by enzymes such as alkaline phosphatase and releasing free technetium from the complexes. In comparison, the novel bone imaging agent, $^{99m}$Tc-labeled 1-hydroxyethyliden-1,1-bisphosphonate (HEDP), demonstrated a notable uptake of bone tissue (Subramanian et al., 1972; Castronovo and Callahan, 1972). The advent of $^{99m}$Tc-labeled methylene bisphosphonate (MDP) and hydroxymethylene bisphosphonate (HMDP), with superior biodistributions compared to [$^{99m}$Tc]Tc-HEDP, has led to these radiopharmaceuticals becoming the preferred

*Corresponding author: mgandomkar@aeoi.org.ir

https://doi.org/10.22034/rpe.2024.467196.1204

agents for bone scanning (Domstad et al., 1980; Mari et al., 1999). The precise structure of these compounds, which are a mixture of short- and long-chain oligomers, remains unknown as they cannot exist as a single species. Furthermore, $^{99m}$Tc-labeled bisphosphonates exhibit prolonged blood clearance, necessitating a delay of 2-6 hours prior to initiating the bone scan (Love et al., 2003).

Rhenium-188, which exhibits exciting characteristics such as a 16.9-hour half-life and a maximum $\beta$-energy of 2.12 MeV, represents a compelling radionuclide for therapeutic applications. Moreover, its 155 keV gamma emission enables the visualization of distribution patterns, thereby facilitating the dosage estimation. Moreover, rhenium-188 is produced using an in-house $^{188}$W/$^{188}$Re generator, as is the $^{99}$Mo/$^{99m}$Tc generator. The analogous chemical properties between technetium and rhenium have resulted in the development of [$^{188}$Re]Re-HEDP, which has demonstrated unexpected characteristics as a bone-seeking agent (Elder et al., 1997). Nevertheless, previous studies have demonstrated that the rhenium release from the radiocomplex leads to unnecessary radiation exposure of other organs, a delay in blood clearance, and a significant gastric uptake (DE WINTER et al., 1999). Therefore, it is necessary to design a novel bone-specific radiopharmaceutical with enhanced potency, stability, and bone accumulation properties. The preparation of a radiopharmaceutical with optimal characteristics, including higher bone absorption, faster blood clearance, and the capacity for earlier imaging post-injection, is a necessity. Such a radiopharmaceutical could confer significant advantages. To achieve this objective, new generations of bisphosphonate tracers have been developed (Erfani et al., 2017). Furthermore, second- and third-generation of $^{99m}$Tc-labeled bisphosphonate analogues have been developed and subjected to investigation (Golshaiyan et al., 2023). In this study, the in vivo comparison of different bisphosphonates labeled with $^{99m}$Tc/$^{188}$Re was conducted to achieve a tracer with high bone uptake.

## 2 Material and Methods

All chemical reagents were purchased from Sigma/Aldrich and used without further purification. Technetium-99m and rehenium-188 were eluted from a commercial $^{99}$Mo/$^{99m}$Tc and $^{188}$W/$^{188}$Re generators (Iran, Tehran, Pars Isotope Co) with saline solution (0.9% NaCl). Radioactivity was determined in a dose calibrator (Isomed, Germany). Quantitative gamma counting was performed using a NaI(Tl) counter.

### 2.1 Radiolabeling with $^{99m}$Tc/$^{188}$Re

For radiolabeling with technetium-99m, solutions of the first and third-generation bisphosphonates (methylene bisphosphonate (5 mg.mL$^{-1}$) and zoledronate (0.15 mg.mL$^{-1}$)) were made by dissolving in the appropriate amounts of water and citrate buffer, respectively. Ascorbic acid (0.2 mg) was mixed in the vials. Additions included 0.1 mg and 0.2 mg of acidic solution of $SnCl_2.2H_2O$ (nitrogen-purged). Then, 185 MBq of technetium-99m was made to the final solution. The pH was adjusted to pH = 3 and pH=6 for zoledronate and methylene bisphosphonate solution, respectively. The final solution was subjected to vigorous shaking for 60 seconds. The subsequent incubation was conducted in a sealed container for 20 minutes at room temperature.

Rehenium-188 radiolabeling was done by preparing a solution containing second-generation bisphosphonate (pamidronate, 5 mg.mL$^{-1}$) in the water. $SnCl_2.2H_2O$ (0.5 mg in nitrogen-purged 0.1 M HCl) and ascorbic acid (2.5 mg) were mixed in a vial. Then, 0.1 mg of potassium perrhenate (as a carrier) was added. Then, the mixture was radiolabeled by 185 MBq of rhenium-188 in 1 mL saline. The pH of the final solution was adjusted using 1N HCl (pH=3), and the solution was incubated at 100 °C for 30 minutes.

### 2.2 Radiochemical analysis

Radiochemical purity analysis of radiolabeled bisphosphonates was carried out by thin layer chromatography method using Whatman 1 strips as stationary phase, and acetone and water solvents as mobile phases. Subsequently, the chromatographic paper stripes were analyzed to determine the radiochemical purity. The acetone solution was used to distinguish the free pertechnetate and perrhenate (Rf=1.0). The water solution visualized the colloidal impurity (Rf=0.0) and pertechnetate, perrhenate, and radiocomplex, which move with the solvent front (Rf=1.0).

The stability of the radiocomplexes was assessed in both saline and human serum. Aliquots were obtained at various intervals after radiolabeling and analyzed using paper chromatography strips. Freshly human serum (0.5 mL) was mixed with 50 $\mu$L of radiolabeled complex, and the mixture was incubated at 37 °C. At various times after the reaction, 50 $\mu$L aliquots were taken out and treated with 50 $\mu$L of ethanol. Serum proteins were precipitated from the samples by centrifuging them for five minutes at 1000 rpm. Paper chromatography was then used to analyze the supernatants.

### 2.3 Biodistribution in mice

The animal studies were conducted under the procedures of our institution and with the generally accepted guidelines for such work. Biodistribution experiments were carried out with an intravenous administration (50 $\mu$L) of radiocomplex solution (1.85 MBq) via a tail vein. Groups of three mice were sacrificed and interested organs were excised, weighed and counted in a $\gamma$-counter. Subsequently, radioactivity uptake in the desired organs was calculated as the percent injected dose per gram tissue (ID/g %).

### 2.4 Imaging

Solutions containing approximately 1.85 MBq of radioactive complexes were administered by intravenous injections into the tail vein of mice. Planar scintigraphy with a single head gamma camera (small area mobile, 140 keV,

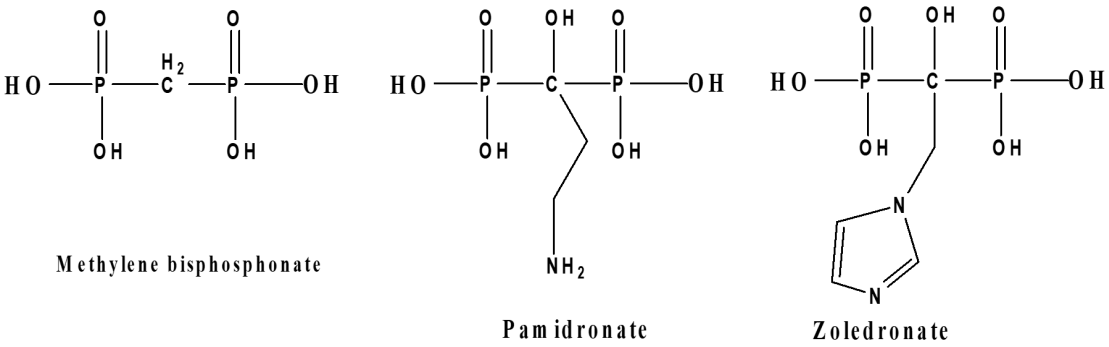


**Figure 1:** Chemical structure of a first, second and third generation bisphosphonates.

Siemens) was used to evaluate the bone uptake of radiocomplexes. Before imaging, intraperitoneal ketamine and xylazine anesthesia were administered to the mice. Then, scanning process was done during 5 minutes at a matrix size of 256 × 256.

## 3 Results and discussion

### *3.1 Radiolabeling*

Technetium-99m bisphosphonates were prepared by decreasing the oxidation state of $[^{99m}Tc]TcO_4^-$ with the aqueous solvent tin chloride (Fig. 1). The amounts of reagents used in the radiolabeling process are important to obtain the best radiolabeling yields. In order to achieve maximum in vivo specific target uptake for the radiolabeled ligand, high specific activity of radiolabeling is required. However, a sufficient amount of ligand must still be present in the formulation to achieve high radiolabeling yield complex formation.

Rhenium-188 was reduced to the lower oxidation state with stannous chloride to allow reaction with the ligand. Compared to technetium, rhenium is more challenging to reduce and tends to return to a higher oxidation state more quickly when reduced (Deutsch et al., 1986). The formation of $SnO_2$ can be achieved through the use of an excess of tin, which subsequently results in a decline in the efficiency of radiolabeling. Ascorbic acid was employed as a stannous ion stabilizer and antioxidant. Colloidal formation of $SnO_2$ at near neutral and elevated pH (above 6) may be the reason for the low radiolabeling yield at high pH. This phenomenon results in elevated liver and spleen uptake and particle trapping within the lung capillaries.

Paper chromatography measurements of radiolabeled bisphosphonates showed that in the acetone system, the Rf values of $[^{99m}Tc]TcO_4^-$ and $[^{188}Re]ReO_4^-$ were 0.9-1.0, while radiolabeled bisphosphonates, $[^{99m}Tc]TcO_2$, and $[^{188}Re]ReO_2$ remained at the bottom. In the water system, $[^{99m}Tc]TcO_4^-$, $[^{188}Re]ReO_4^-$, and radiolabeled bisphosphonates were in Rf = 0.8-1.0, while $[^{99m}Tc]TcO_2$ and $[^{188}Re]ReO_2$ remained at the bottom. The radiolabeling efficiency of radiolabeled bisphosphonates was > 90% in paper chromatography (Fig. 2). It is crucial for radioconjugates that isotopic chelation stays steady over time. The paper chromatography analyses showed that the radiolabeled bisphosphonates showed > 90% radiochemical purity in saline and serum for 6 hours.

### *3.2 Animal study*

Biological evaluations of radiolabeled bisphosphonates were performed in mice. The results are shown in Table 1 and Fig. 3. As results showed, all radiocomplexes mainly accumulated in the bone, kidneys and liver. In the bone at high level of uptakes including 3.86 ± 0.12 %ID/g, 4.19 ± 0.15 %ID/g and 0.74 ± 0.20 %ID/g were observed for $[^{99m}Tc]$Tc-methylene bisphosphonate, $[^{99m}Tc]$Tc-zoledronate and $[^{188}Re]$Re-pamidronate, respectively (2 h post injection). No significant concentration of radiocomplexes in any other organs were obtained.

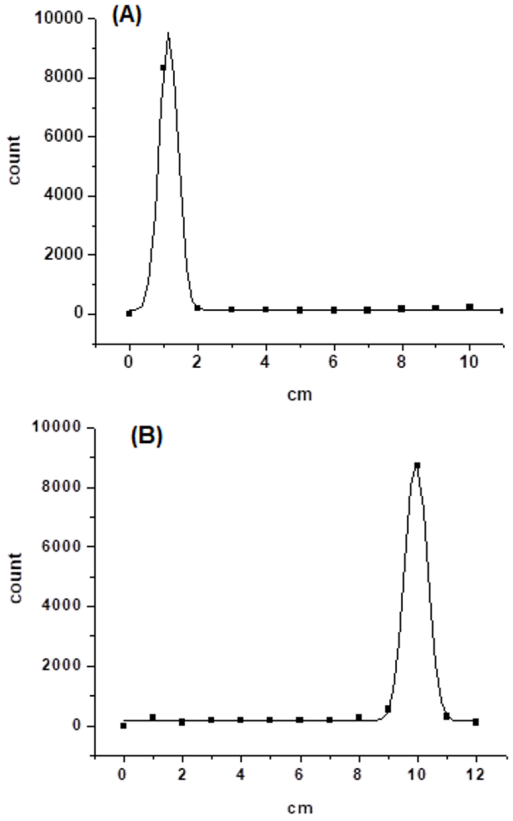


**Figure 2:** Quality control of radiolabeled bisphosphonates by paper chromatography in acetone (A) and water (B) as solvents.

**Table 1:** Biodistribution of radioactivity after intravenous administration of radiolabeled bisphosphonates in normal mice at 2 h post injection (%ID/g ± SD, n=3).

| Organs | Radiolabeled bisphosphonates | | |
|---|---|---|---|
| | [$^{99m}$Tc]Tc-methylene bisphosphonate | [$^{99m}$Tc]Tc-zoledronate | [$^{188}$Re]Re-pamidronate |
| Blood | 0.66 ± 0.13 | 0.60 ± 0.10 | 0.23 ± 0.05 |
| Kidneys | 1.72 ± 0.08 | 2.65 ± 0.12 | 1.54 ± 0.22 |
| Stomach | 0.29 ± 0.05 | 0.17 ± 0.08 | 0.85 ± 0.06 |
| Intestine | 0.43 ± 0.02 | 0.46 ± 0.05 | 0.60 ± 0.15 |
| Thyroid | 0.26 ± 0.13 | 0.20 ± 0.14 | 0.15 ± 0.05 |
| Liver | 1.19 ± 0.27 | 0.36 ± 0.10 | 5.80 ± 1.12 |
| Bone | 3.86 ± 0.12 | 4.19 ± 0.15 | 0.74 ± 0.20 |

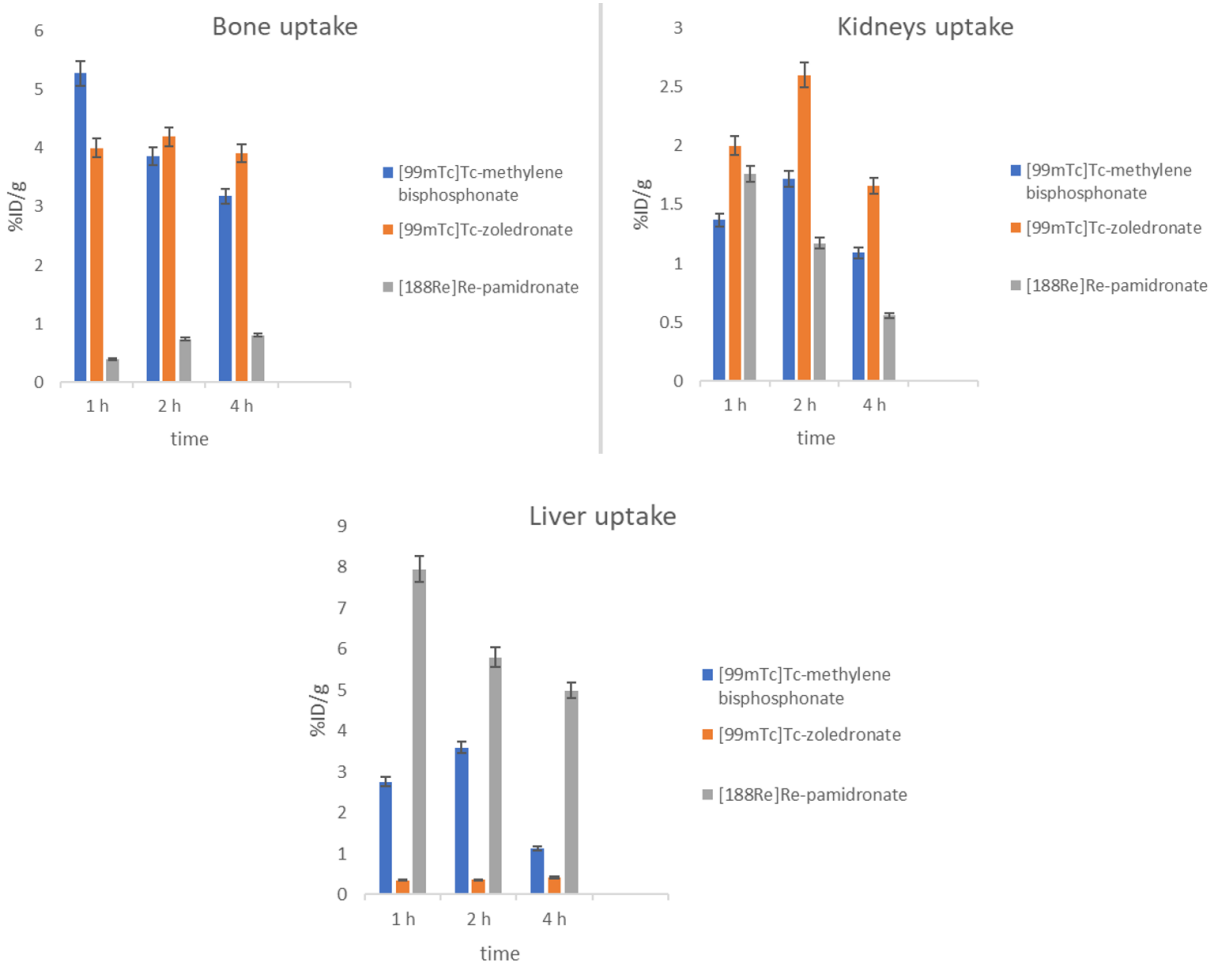


**Figure 3:** Comparison of bone, kidneys and liver uptake for radiolabeled bisphosphonates at different time after injection.

Based on the comparison results shown in Fig. 3, while [$^{99m}$Tc]Tc-methylene bisphosphonate showed rapid accumulation of activity in bone, [$^{99m}$Tc]Tc-zoledronate also showed significant bone uptake. The liver uptake of [$^{99m}$Tc]Tc-methylene bisphosphonate was greater than that of [$^{99m}$Tc]Tc-zoledronate, while the kidneys uptake was less pronounced. The results of biodistribution studies showed bone uptake for [$^{188}$Re]Re-pamidronate and significant liver uptake.

According to the results, low blood activity uptake and fast blood clearance are the advantages of these radiocomplexes. It should also be noted that the significant kidneys uptake indicates renal excretion of these radiocomplexes and that the kidneys play a significant role in their elimination. The remaining radioactivity in bone over time could be due to a duo-binding with excellent retention and slow release. The significant liver uptake for [$^{188}$Re]Re-pamidronate may be explained by the aliphatic chain in the compound structure and the pharmacokinetic properties of the prepared rhenium bisphosphonate chelate. As shown in Fig. 3, the main advantages of [$^{188}$Re]Re-pamidronate were the retention of activity in bone and the decrease in liver uptake with time after injection.

Scintigraphy showed that the radiocomplexes accumulated mainly in the skeleton and kidneys (Fig. 4). Clear images of the mouse skeleton were obtained, supporting

the results of the biodistribution studies. However, the quality of the [$^{188}$Re]Re-pamidronate image was significantly poor, presumably due to photons of high-energy gamma rays in a scattered state (i.e., 478, 633, 829, and 913 keV). As a result, more activity and higher contrast were required to achieve the same hot sphere detection capability compared to technetium-99m imaging.

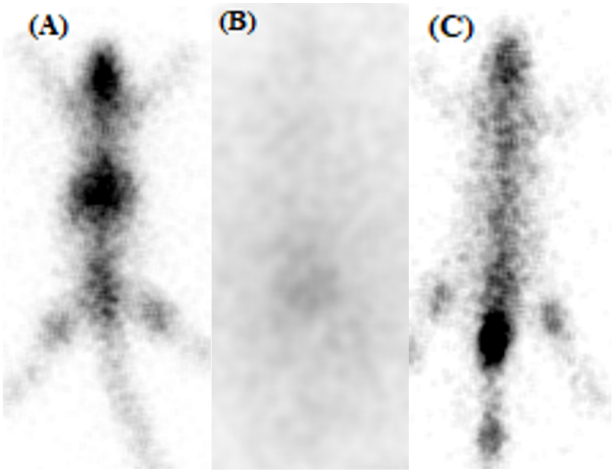


**Figure 4:** Anterior image of radiocomplexes ((A) [$^{99m}$Tc]Tc-methylene bisphosphonate, (B) [$^{188}$Re]Re-pamidronate and (C) [$^{99m}$Tc]Tc-zoledronate) in mice.

## 4 Conclusions

These results suggest that the addition of a hydroxyl group to the central carbon is effective in increasing bone uptake. In addition, another side chain attached to the carbon may be affected not only in the cellular but also in the physicochemical action of the bisphosphonate. Furthermore, the results indicate that to assess the adsorption and retention of diphosphonate on hydroxyapatite, it is essential to consider the entire molecular structure which influenced by both the side chain structures.

## Acknowledgements

We greatly appreciate the supports of this work by Nuclear Science and Technology Research Institute, Tehran, Iran.

## Conflict of Interest

The authors declare no potential conflict of interest regarding the publication of this work.

## References

Castronovo, F. P. and Callahan, R. J. (1972). New bone scanning agent: $^{99m}$Tc-labeled 1-hydroxy-ethylidene-1, 1-disodium phosphonate. *Journal of Nuclear Medicine*, 13(11):823–827.

DE WINTER, F., Brans, B., VAN DE WIELE, C., et al. (1999). Visualization of the stomach on rhenium-186 HEDP imaging after therapy for metastasized prostate carcinoma. *Clinical Nuclear Medicine*, 24(11):898.

Deutsch, E., Libson, K., Vanderheyden, J.-L., et al. (1986). The chemistry of rhenium and technetium as related to the use of isotopes of these elements in therapeutic and diagnostic nuclear medicine. *International Journal of Radiation Applications and Instrumentation. Part B. Nuclear Medicine and Biology*, 13(4):465–477.

Domstad, P., Coupal, J., Kim, E., et al. (1980). $^{99m}$tc-hydroxymethane diphosphonate: a new bone imaging agent with a low tin content. *Radiology*, 136(1):209–211.

Elder, R., Yuan, J., Helmer, B., et al. (1997). Studies of the structure and composition of rhenium- 1, 1-hydroxyethylidenediphosphonate (HEDP) analogues of the radiotherapeutic agent $^{186}$ReHEDP. *Inorganic Chemistry*, 36(14):3055–3063.

Erfani, M., Rahmani, N., Doroudi, A., et al. (2017). Preparation and evaluation of rhenium-188-pamidronate as a palliative treatment in bone metastasis. *Nuclear Medicine and Biology*, 49:1–7.

Fleisch, H., Russell, R., and Straumann, F. (1966). Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. *Nature*, 212(5065):901–903.

Golshaiyan, S., Erfani, M., Shamsaei, M., et al. (2023). An optimized formulation for [$^{99m}$Tc] Tc radiolabeling of zoledronic acid as bone imaging agent. *Iranian Journal of Nuclear Medicine*, 31(2):137–143.

King, M. A., Weber, D. A., Casarett, G. W., et al. (1980). A study of irradiated bone. Part II. changes in Tc-99m pyrophosphate bone imaging. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, 21(1):22–30.

Love, C., Din, A. S., Tomas, M. B., et al. (2003). Radionuclide bone imaging: an illustrative review. *Radiographics*, 23(2):341–358.

Mari, C., Catafau, A., and Carrio, I. (1999). Bone scintigraphy and metabolic disorders. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 43(3):259.

Russell, R. G., Mühlbauer, R., Bisaz, S., et al. (1970). The influence of pyrophosphate, condensed phosphates, phosphonates and other phosphate compounds on the dissolution of hydroxyapatite in vitro and on bone resorption induced by parathyroid hormone in tissue culture and in thyroparathyroidectomised rats. *Calcified Tissue Research*, 6:183–196.

Russell, R. G. G. (2006). Bisphosphonates: from bench to bedside. *Annals of the New York Academy of Sciences*, 1068(1):367–401.

Subramanian, G., McAfee, J., Blair, R., et al. (1972). $^{99m}$Tc-EHDP: A potential radiopharmaceutical for skeletal imaging. *Journal of Nuclear Medicine*, 13(12):947–950.

Valdez, V. A. and Jacobstein, J. G. (1980). Decreased bone uptake of technetium-99m polyphosphate in thalassemia major. *Journal of Nuclear Medicine*, 21(1):47–49.


**To cite this article:**

Erfani, M. , Mikaeili, A. , Goudarzi, M. , Fallah, Z. and Rezaei, M. (2025). Comparison of radiolabeled bisphosphonates in bone metastasis. Radiation Physics and Engineering, 6(2), 41-46. doi: 10.22034/rpe.2024.467196.1204

DOI: 10.22034/rpe.2024.467196.1204

To link to this article: https://doi.org/10.22034/rpe.2024.467196.1204