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Production, quality control, and bio-distribution studies of ^{159}Gd-EDTMP as a palliative agent for bone pain

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Abstract

Introduction: Particle-emitting, bone-seeking radiopharmaceuticals have attracted the attention of the nuclear medicine community over the last three decades for the treatment of the pain of osteoblastic metastases. The objectives of this research were to produce quality-controlled ^{159}Gd-EDTMP in order to provide a new therapeutic radiopharmaceutical for use in clinical applications.

Methods: The investigation was an experimental study in which ^{159}Gd (T1/2=18.479 h, Eβ (max)=970.60 keV, Eγ=363.55 (11.4%) keV] was produced by thermal neutron bombardment of natural Gd_2O_3 at the Tehran Research Reactor (TRR) for a period of 7 d at a flux of 3–4×10^{13} neutrons/cm^2.s. It was then quality-controlled and used to radio-label the in-house prepared ethylene diamine tetra acetic acid (EDTM).

Results: Complexation parameters were optimized to achieve maximum yields (>99%). The radiochemical purity of ^{159}Gd-EDTMP was checked by radio thin layer chromatography RTLC. It was found to retain its stability at room temperature (>95%). Bio-distribution studies of the complexes conducted in wild rats showed significant bone uptake with rapid clearance from blood.

Conclusion: The properties of the ^{159}Gd-EDTMP that was produced suggest then use of a new, efficient, palliative therapeutic agent for metastatic bone pain instead of some other current radiopharmaceuticals.

Keywords: EDTMP, gadolinium, radiotherapy, bone

1. Introduction

Bone metastases are a frequent complication of cancers such as prostate and breast (70% of patients), lung, colon, stomach, bladder, uterus, rectum, thyroid, or kidney (15-30% of patients) cancers leading to painful and untreatable consequences including fractures, hypercalcemia, and bone pain, as well as reduced performance status and quality of life. The exact incidence of bone metastasis is unknown, but it is estimated that 350,000 people die with bone metastases annually in the United States (1, 2). For all these reasons, bone metastasis is a serious and costly complication of cancer. Currently, the treatment of bone pain remains palliative at best with systemic therapy (analgesics, hormones, chemotherapy, steroids, and bisphosphonates) as well as local treatments (such as surgery,

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nerve blocks, and beam radiation) (3). Particle-emitting bone-seeking radiopharmaceuticals have attracted the attention of the nuclear medicine community over the last three decades for the treatment of the pain of osteoblastic metastases. For the eight pharmaceuticals including: ^{188}Re (Sn) HEDP, ^{153}Sm-EDTMP, ^{90}Y-Citrate, ^{186}Re(Sn)HEDP, ^{117m}Sn-DTPA, ^{32}P-phosphate, ^{89}Sr-chloride, ^{85}Sr-chloride, there are published data on clinical trials in humans. All are reactor produced, and all emit a beta particle except for tin (Sn)-117 pentetate and strontium-85 (Sr-85) which produce low energy conversion electrons. $^{89}SrCl_2$ and ^{153}Sm-EDTMP are widely preferred for the management of pain arising due to skeletal metastasis. $^{89}SrCl_2$ has the advantage of comparatively longer half-life of 50.5 days, which makes it possible to supply the radiopharmaceuticals worldwide. ^{153}Sm-EDTMP is preferred by many investigators due to the more favorable radionuclidic properties of ^{153}Sm. However, the relatively short half-life of ^{153}Sm precludes its use from places other than proximal or well connected to the production site (4-12). ^{177}Lu-EDTMP or other phosphonates are also proposed as alternatives to ^{153}Sm-EDTMP as the long half-life (13-18).

The ^{159}Gd radionuclide is a beta ($E_{\beta(max)}$=970.60 keV(32%)) and gamma (main energy: 363.55 keV) emitter with a half-life of 18. 479 h (19). The physical characteristics of the ^{159}Gd isotope suggest that it has the potential to be used in nuclear medicine research (20-22). The β^- energy of ^{159}Gd is lower than that of ^{89}Sr and hence the bone marrow dose is expected to be much lower. The presence of accompanying gamma photons which can be imaged by using widely available gamma camera systems is advantageous in carrying out simultaneous dosimetry and scintigraphy studies. ^{159}Gd can be produced by a relatively easy route involving thermal neutron bombardment on natural Gd_2O_3 in medium flux research reactors. The requirement for an enriched target does not arise and radionuclidic impurities are not formed by radiative capture during neutron activation. G. J. Beyer introduced the ^{159}Gd-ethylenediaminetetra-methylinephosphonic acid agent for effective palliative treatment of skeletal metastases (23). These types of phosphonate complexes concentrate in the skeleton, in proportion to osteoblastic activity and interrupt the vicious cycle and cause not only a reduction in osteolytic bone lesions, but also decrease the tumour burden in bone (2). In the present study, the preparation, quality control and biodistribution studies of ^{159}Gd-EDTMP is reported in order to provide a new therapeutic radiopharmaceutical to enter in clinical applications in the country.

2. Material and Methods

2.1. Materials

Gadolinium oxide (spectroscopic grade N99.99% pure) was obtained from E. Merck (Darmstadt, Germany). EDTMP was synthesized and characterized in-house as per the reported procedure. All other chemicals were purchased from Sigma-Aldrich Chemical Co. U.K. Whatman 3 MM chromatography paper (UK) was used as the stationary phase. Radiochemical purity of gama-spectroscopy on the base of 363.55 keV peak and beta-spectroscopy were carried out using the HPGe detector and the Wallac 1220 Quantulus liquid scintillation spectrometer, respectively. Radio-chromatography was performed by counting of Whatman No. 2 using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edition.

2.2 Synthesis of EDTMP

EDTMP was synthesized by following a Mannich-type reaction (24), using orthophosphorus acid, 1,2-ethylenediamine and formaldehyde in strongly acidic medium. In a typical reaction, 1,2-ethylenediamine (5 g, 0.08 mol) was added slowly to a solution of anhydrous orthophosphorus acid (33.66 g, 0.34 mol), in concentrated HCl (33.44 g, 0.92 mol) and the mixture was allowed to reflux. Formaldehyde 37% (10 g, 0.01 mol) was added drop wise over a period of 15 min to the fluxing mixture. The refluxing was continued for another 2 h and subsequently the mixture was cooled to room temperature overnight. Then the resultant was added to ethanol and EDTMP was precipitated in ethanol. The precipitation then was filtered under vacuum and then was dried in oven in 60 ℃. It was purified after recrystallization from water/methanol m.p. 214–215 ℃. IR (KBr, ν cm^{-1}): 3308, 2633, 2311, 1668, 1436, 1356. ^{1}H-NMR (D_2O, δ ppm): 3.53 (d, J=12.3 Hz, 8H,-N-CH_2-P=O), 3.85 (s, 4H, -N-CH_2-). ^{13}C NMR (D_2O, δ ppm): 51.63, 52.73. ^{31}P NMR (D_2O, δ ppm): 10.52 (25).

2.3 Production of ^{159}Gd

^{159}Gd was produced by thermal neutron bombardment on natural Gd_2O_3 at the Tehran Research Reactor (TRR) for a period of 7 d at a flux of $3–4\times10^{13}$ neutrons/cm^2.s. In a typical procedure, 11.52 mg of Gd_2O_3 was sealed and irradiated in the reactor after placing it inside aluminum can. The irradiated powder was dissolved 1 mL of HCl

0.1M heated until all the powder was completely dissolved. This radiochemical form was used for the subsequent studies. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as HPGe spectroscopy for the detection of various interfering beta and gamma emitting radionuclides.

2.4 Preparation ^{159}Gd -EDTMP complex

A stock solution of EDTMP was prepared by dissolving EDTMP (100 milligram) $NaHCO_3$ buffer (5 ml, pH .9). A portion of this solution containing 37.5 mg of EDTMP, was used for complexation of ^{159}Gd (1 mg, 2 mCi) which results in a complex with specific activity of 12.3GBq/mmol. The pH of the reaction mixture was adjusted to 7 and was incubated at room temperature for 15 min to facilitate complexation. The radiochemical purity of the preparation was determined by paper chromatography using two solvent systems. Ammonia/methanol/water (2:20:40 v/v) and also 1 mM DTPA were used as the eluting solvents for paper chromatography.

2.5 Stability of ^{159}Gd -EDTMP in final formulation

The final formulation was stored in 25 ℃ for two days in order to determine the stability. Radiochemical purity of the complex was studied by frequent ITLC analysis using the mentioned system.

2.7 Stability of ^{159}Gd -EDTMP in the presence of human serum

To determine the stability of final formulation in human serum, 200 µCi (200 µl) of complex (^{159}Gd -EDTMP) was incubated in the freshly prepared human serum (300 µl) at 37 ℃ for 2 d. The stability was determined by performing frequent ITLC analysis using the mentioned system.

2.8 Biodistribution studies in rats

Biodistribution studies of the ^{159}Gd -EDTMP complex were carried out in wild-type rats each weighing 180–210 g. A volume of 200 µl containing 200±5 µCi of radioactivity was injected through a lateral tail vein. The animals were sacrificed at the exact intervals of 2 h, 4 h, 6 h, 20h and 40 h post injection. The tissue and the organs were excised and the activity associated with each organ/tissue was measured in a flat-type NaI (Tl) scintillation counter. The uptake in different organs/tissue was calculated from these data and expressed as % Injected Dose (% ID/gram).

3. Results

3.1 Production and quality control of ^{159}Gd

Irradiation of natural Gd_2O_3 was performed at a thermal neutron flux of $3–4\times10^{13}$ neutrons/cm^2.s for 7 d at TRR and the radionuclide was prepared according to regular methods with a range of specific activity 15-20 mCi/mg for radiolabeling use. Gamma ray spectrum of the appropriately diluted $^{159}GdCl_3$ solution showed a major peak at 363.5 keV, which are the photo-peak of ^{159}Gd (Figure 1).

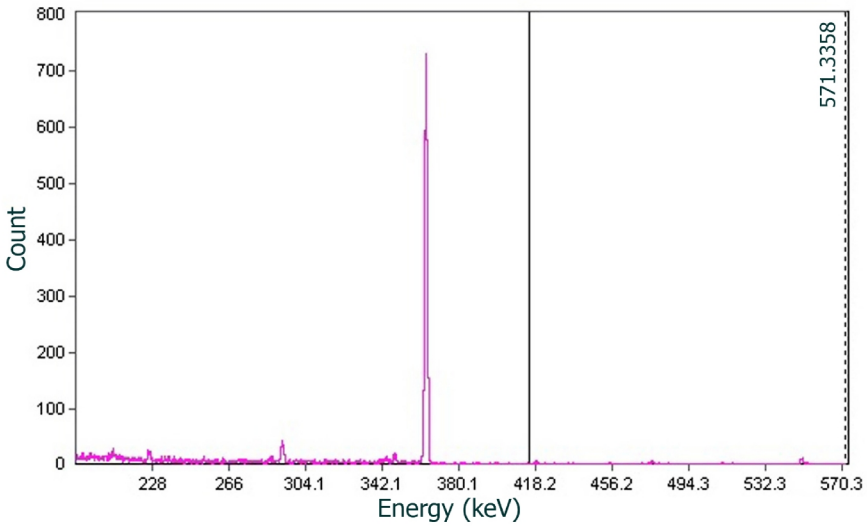

Figure 1. γ-ray spectrum for 159Gd chloride solution

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtering. The absence of any other photo-peaks in the gamma ray spectrum indicated that the ^{159}Gd was produced with a radio-nuclidic purity of>99.99%. The radiochemical purity of the ^{159}Gd solution was checked in two solvent systems, including DTPA 1 mM and ammonia/methanol/water (2:20:40 v/v) and Whatman 3MM as the stationary phase. In the first solvent system, Gd^{3+} cations migrated to the higher R_f. The ITL-chromatogram is presented in Figure 2a. In the latter, the Gd^{3+} cations remained at the point of spotting (lower R_{fs}), as shown in Figure 2b.

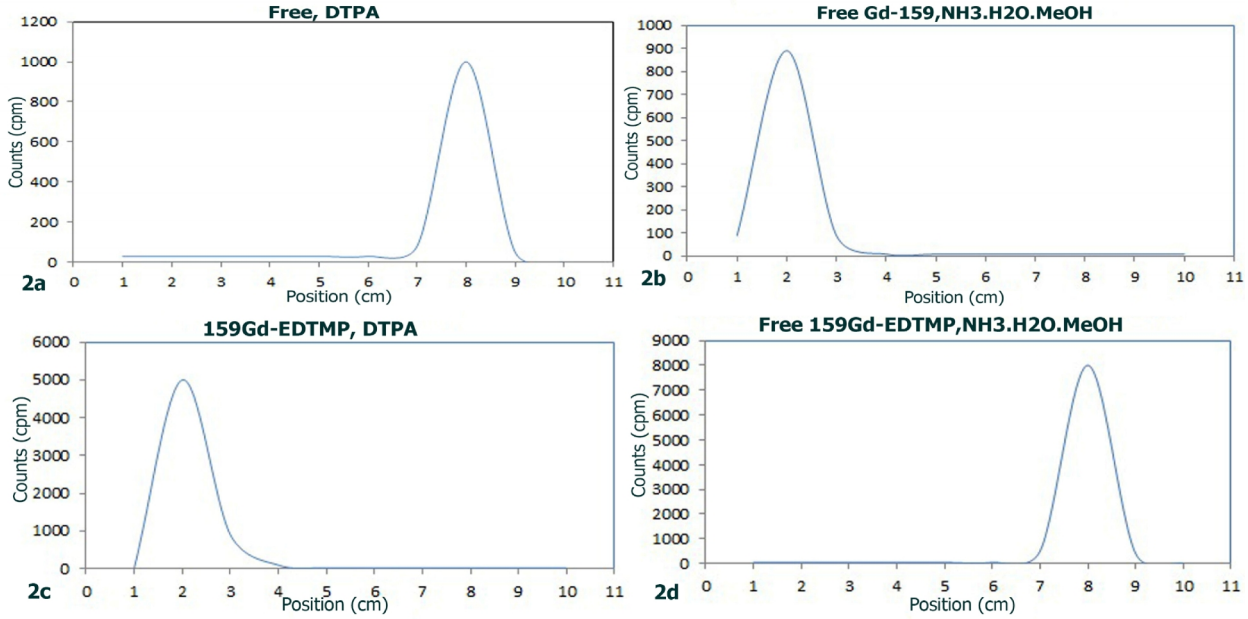

Figure 2. TLC chromatogram of 159GdCl3solution in DTPA, Whatman 3 MM system (a) and 159GdCl3solution in NH4OH/MeOH/HOH: 2/20/40, Whatman 3 MM (b), 159Gd-EDTMP solution in DTPA, Whatman 3 MM system (c), 159Gd-EDTMP solution in NH4OH/MeOH/HOH: 2/20/40

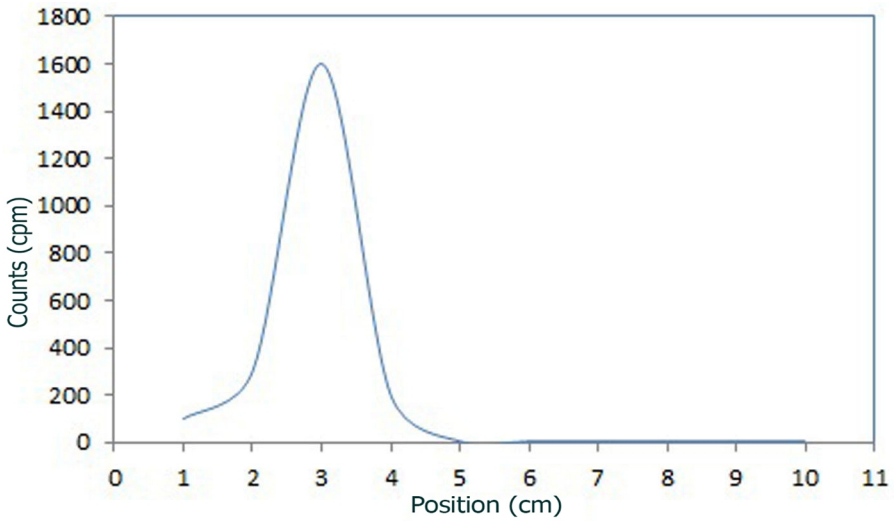

Figure 3. TLC chromatogram of 159Gd -EDTMP complex after 48 hin DTPA, Whatman 3 MM system

3.2 Preparation of ^{159}Gd-EDTMP complex

Various parameters such as ligand concentration, temperature, pH of reaction and time were varied in order to reach the maximum complexation. It was observed that complexation gradually increased with increase in ligand

concentration and reached to ~ 100% at a ratio of [ligand]/[metal] ~ 15:1. On variation of reaction pH from 4 to 10, it was found that a maximum complexation yield of >99% was achieved in the pH range of 6 to 8. The *in vitro* stability studies were performed by incubating the complex at room temperature and showed that the radiochemical purity of the complex remained >95% up to 4 days after preparation. In paper chromatography using (DTPA 1 mM as solvent and Whatman 3 MM as stationary phase), it was observed that the un-complexed ^{159}Gd moved towards the solvent front (R_f=0.8–0.9) while the ^{159}Gd -EDTMP complex remained at the point of spotting (R_f=0-0.1) under identical conditions (Figure 2a and 2b). Paper chromatography using another system, ammonia/methanol/water (2:20:40 v/v), was also performed. It was observed that the ^{159}Gd -EDTMP complex moved towards the solvent front (R_f=0.8-0.9) while the un-complexed ^{159}Gd remained at the point of spotting (R_f=0-0.1) under identical conditions (Figure 2c and 2d). The stability of ^{159}Gd -EDTMP complex was checked up to 48 h (Figure 3) using DTPA 1 mM as solvent and Whatman 3 MM as stationary phase after preparation. The complex was stable in final sample and its radiochemical purity was above 99% even 48 h after preparation using Whatman 3 MM eluted with 1 mM solvent of DTPA. The latter stability test was developed for the complex in presence of human serum at 37 ℃ using ITLC (DTPA 1 mM as solvent and Whatman 3 MM as stationary phase) (Figure 4).

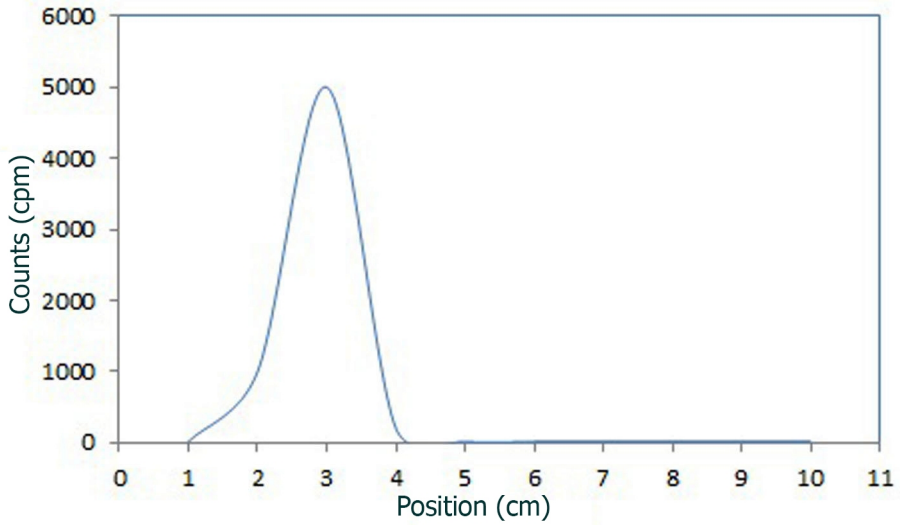

Figure 4. TLC chromatogram of 159Gd -EDTMP complexin presence of human serum at 37 ˚Cin DTPA, Whatman 3 MM system

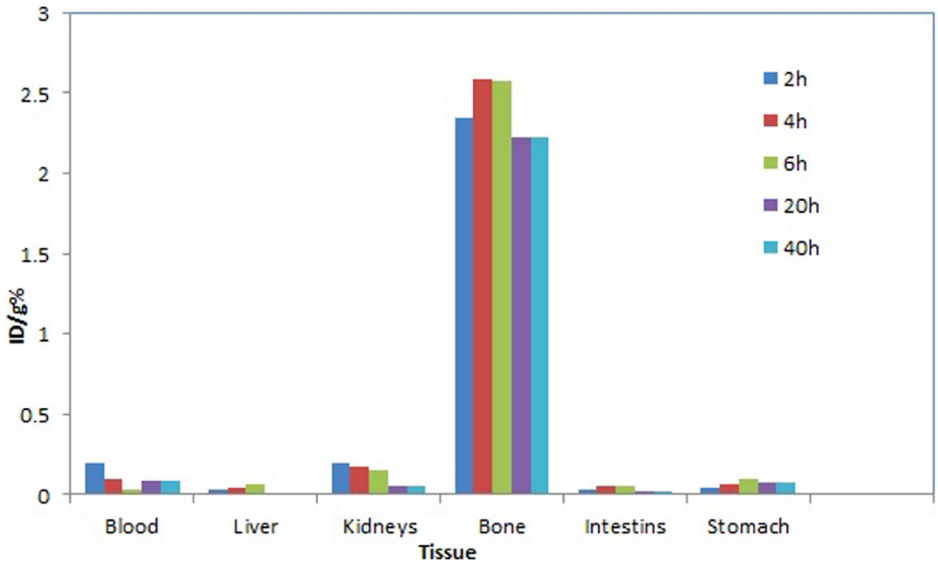

Figure 5. Percentage of injected dose per gram (ID/g %) of 159Gd -EDTMP in wild-type rat tissues at 2 h, 4 h, 6 h, 20 h and 40h post injection

3.3 Biodistribution studies in rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (2 h, 4 h, 6 h, 20 h and 40h). Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lungs and skin samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g). The distribution of injected dose in rat organs up to 40 hours after injection of ^{159}Gd-EDTMP (200 μCi / 200 μl) solution was determined. Based on these results, it was concluded that the major portion of injected activity was extracted from blood circulation into bones. The results of the biodistribution studies are expressed in Figure 5 and revealed significant uptake in skeleton within less than 4 h pi. It was determined in wild-type animals for better comparison for 2–40 h pi. The blood wash-out happens after 4h. ^{159}Gd-EDTMP is rapidly taken up in bones 2 h after administration and retains almost constantly up to 40 hours while the free radio-lanthanides ($^{zzz}Ln_s$) uptake increases at first as a result of affinity of the lanthanide ions to the bone due to their similarity to the calcium cation. It reaches a maximum value more than that of ^{zzz}Ln-EDTMP (chelated radio-lanthanide) and then decreases to a value much less than that of ^{zzz}Ln-EDTMP. ^{159}Gd-EDTMP has almost no accumulation in liver, while as a free cation, being transferred by serum metalloproteins, free Gd-159 would accumulate in liver. Also ^{159}Gd-EDTMP has almost no accumulation in spleen, while free Gd-159 would accumulate in spleen especially after 2 d.

4. Discussion

4.1 Production and quality control of ^{159}Gd

The absence of any other photo-peaks in the gamma ray spectrum indicated that the radio-gadolinium was prepared with a radionuclidic purity of more than 99.99%. As the Gd^{3+} cations are complexed to the more lipophilic ^{159}Gd-DTPA form, migration to higher R_f occurs. As expected, the data obtained from other solvent systems concluding (ammonia/methanol/water: 2/20/40 v/v) confirmed that there was just one cationic specimen in the sample. In the second system, lower R_f was observed because of the significant difference in polarity between the Gd(III) cation and the solvent system it remains at the origin of the stationary phase.

4.2 Preparation of the ^{159}Gd-EDTMP complex

As a result of the similarity between the polarities of the ^{159}Gd-EDTMP complex and the ammonia/methanol/water solvent system, the more lipophilic species ^{159}Gd-EDTMP complex (other than Gd^{3+}) moved towards the solvent front in the (ammonia/methanol/water: 2/20/40 v/v) - Whatman 3 MM stationary phase system and vice versa for the other solvent system.

4.3 Biodistribution studies in rats

The affinity of the phosphonate complex is more than that of the free ion to the bone (4-7); therefore the free cation is released from the bone structure faster than ^{zzz}Ln-EDTMP. As mentioned earlier, ^{159}Gd-EDTMP is rapidly taken up in bones and the trapping continues in a way that almost no blood circulation activity as well as kidney excretion can be observed. As can be seen from Figure 5, and similar to the previous studies (3-17, 25), the washed-out activity of free cation is higher than that of complexed isotope. One of the most important features of the prepared formula was that there was almost no accumulation of ^{159}Gd-EDTMP in the spleen or liver, which is a major advantage for its use as a therapeutic radiopharmaceutical because it would be possible to increase the maximum injectable dose (25). Another advantage of the prepared compound is that its activity was observed to be retained in the skeletal bones until 40 h post injection up to which time the bio-distribution studies were continued.

5. Conclusions

^{159}Gd-EDTMP preparation (radiochemical purity of more than 99%) was administered to normal rats and related biodistribution data were checked 2 h to 40 hours later showing at least 2.3 ID%/g accumulation of the drug in the bone tissues. Also ^{159}Gd-EDTMP has almost no accumulation in liver and spleen which is main advantage of this radiopharmaceutical. The development of other ^{159}Gd-labeled therapeutic molecules, monoclonal antibodies and also peptides for ultimate radioimmunotherapy is possible.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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