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Original Article

Preliminary Dosimetry Study of ^{67}Ga-AATS for Human Based on Biodistribution Data in Rats

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Abstract

Introduction
Gallium-67 (^{67}Ga) has been used as a radionuclide for imaging a variety of solid tumors since 1969. Since then use of various gallium-based radiotracers has been reported. Recently, ^{67}Ga-labeled acetylacetate bis(thiosemicarbazones) (^{67}Ga-AATS) complex with significant tumor accumulation and fast blood clearance has been employed.

Materials and Methods
In this study, the absorbed dose of ^{67}Ga-AATS in each human organ was evaluated and compared with ^{67}Ga-citrate as the most commonly used form of ^{67}Ga in nuclear medicine. ^{67}Ga was produced via ^{68}Zn(p,2n)^{67}Ga reaction at 30 MeV cyclotron. Moreover, ^{67}Ga-AATS was produced by adding 50 µl of AATS to absolute ethanol (1 mg/ml) in a gallium-containing vial at 80-90 $^{\circ}$C. The absorbed dose of each human organ was calculated, using RADAR method, based on biodistribution data in Wistar rats.

Results
According to the results, ^{67}Ga-AATS was produced with radionuclidic and radiochemical purity higher than 99% and 93%, respectively. The highest absorbed dose was reported in the bone surface (0.401 mGy/MBq), whereas the whole-body absorbed dose was 0.092 mGy/MBq.

Conclusion
The absorbed dose of each human organ was comparable with the absorbed dose received by each organ after ^{67}Ga-citrate injection. Considering this interesting finding and the significant tumor uptake, it seems that ^{67}Ga-AATS can be used as an appropriate SPECT tracer.

Keywords: Gallium-67, Radiation Dose, Dosimetry

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1. Introduction

Over the past few decades, a large number of experimental studies have evaluated medical radioisotope production [1]. Today, with significant advances in radioisotope production, radiopharmaceuticals play an important role in diagnostic and therapeutic measures for various cancers [2]. However, the sensitivity of tumor-imaging procedures mainly depends on the considerable affinity of radiopharmaceuticals for malignant tissues, compared to normal tissues. Also, the physical properties of radionuclides are regarded as the first parameter which should be considered.

The suitable physical properties and availability of gallium-67 (^{67}Ga) contribute to its significance in radiopharmaceutical research [3]. While this radionuclide has been used for imaging a variety of solid tumors since 1969, it has shown great applicability in the management of patients with lymphoma, as well. So far, various gallium-based radiotracers have been reported [4-6] including acetoacetate gallium-67 complex as a potential radiopharmaceutical [7].

Thiosemicarbazone gallium complexes have shown interesting in vitro and in vivo anti-proliferative activities [8]. Generally, traditional bis-thiosemicarbazones such as diacetyl-bis(N4-methylthiosemicarbazone) (ATSM) and pyruvaldehyde-bis(N4-methylthiosemicarbazone) (PTSM) do not form complexes with gallium due to various chemical and molecular orbital considerations. However, the use of ^{67}Ga-labeled acetylacetate bis(thiosemicarbazones) (^{67}Ga-AATS) complex has been recently reported in imaging procedures [9]. This complex with significant tumor accumulation and fast blood clearance can be considered as a potential SPECT radiotracer for imaging malignancies.

One of the important parameters in developing new radiopharmaceuticals is the dose delivered to non-target organs. In fact, any extra radiation dose can damage healthy tissues and result in serious complications. It is generally accepted that even a 10% reduction in patient dose is of high significance [10].

The main aim of patient dosimetry is the evaluation of risks associated with the administration of radiopharmaceuticals and maximum measures which need to be considered [11]. Today, in nuclear medicine, the most commonly used method for the calculation of internal dose estimates is the Radiation Dose Assessment Resource (RADAR) method [12].

While ^{67}Ga-AATS with significant tumor accumulation has been successfully produced, evaluating the absorbed dose of this complex by each human organ is the next step for introducing this complex as a new radiopharmaceutical for diagnostic purposes. Therefore, in this study, the absorbed dose of ^{67}Ga-AATS in each human organ was evaluated, based on biodistribution data in rats, using RADAR method. Furthermore, since ^{67}Ga citrate is the most commonly used form of ^{67}Ga in nuclear medicine and is known as a good diagnostic agent in patients with lymphoma (or other malignancies), the absorbed dose of ^{67}Ga-AATS was compared with ^{67}Ga-citrate absorption.

2. Materials and Methods

Enriched zinc-68 chloride with a purity of more than 95% was obtained from the Ion Beam Separation Group. ^{67}Ga was produced using 30 MeV cyclotron by the Nuclear Medicine Research Group (Cyclone-30, IBA, Belgium). Other chemicals were purchased from Aldrich Chemical Co. (Aldrich, Germany) and the ion-exchange resins were acquired from Bio-Rad Laboratories (Canada).

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian instrument (FT-80, 80MHz, Varian, France) with tetramethylsilane as the internal standard. The infrared spectrum was measured on a Perkin-Elmer 781 spectrometer, using a KBr disc. Also, mass spectrum was recorded by a Finnigan Mat TSQ-70 spectrometer. Moreover, thin layer chromatography (TLC) for cold compounds was performed on polymer-backed silica gel (F 1500/LS 254,

20*20 cm, TLC Ready Foil, Schleicher & Schuell, Germany).

Normal saline and sodium acetate used for labeling had high purity and were passed through 0.22 mm Cativex filters. Instant TLC (ITLC) was performed by counting Whatman No. 2 papers, using a TLC scanner (Bioscan AR2000, Bioscan Europe Ltd., France).

Analytical high-performance liquid chromatography (HPLC), used to determine the specific activity of the samples, was performed by Shimadzu LC-10AT, armed with two detector systems, a flow scintillation analyzer (Packard-150 TR) and a UV-visible spectrophotometer (Shimadzu), using a Whatman Partisphere C-18 column, 250 × 4.6 mm (Whatman, NJ, USA).

Biodistribution data were acquired by counting normal saline-washed tissues after weighing them on a Canberra High-purity Germanium (HPGe) detector (model GC1020, 7500SL); radionuclidic purity was evaluated by the same detector. For the activity measurement of the samples, CRC Capintec Radiometer (NJ, USA) was employed.

All calculations and ITLC counts were based on 184 keV peak. Animal studies were performed in accordance with the guidelines proposed by the United Kingdom Biological Council on the Use of Living Animals in Scientific Investigations (2nd edition).

1.1. Production and quality control of $^{67}GaCl_3$

^{67}Ga was produced, using 30 MeV cyclotron by the Nuclear Medicine Research Group. Also, ^{68}Zn(p,2n)^{67}Ga was used as the best nuclear reaction for the production of ^{67}Ga. Other impurities were removed during the radiochemical separation process. The final ^{67}Ga was assessed in terms of any possible radionuclidic or radiochemical impurity.

1.2. Production of ^{67}Ga-AATS

AATS was prepared by making slight modifications in the reported method [13]. The labeling procedure was performed according to the previously mentioned method [9]. Briefly, 50 µl of AATS was added to absolute ethanol (1 mg/ml) in a gallium-containing vial and vortexed at 80-90 °C for 30 minutes. The active solution was evaluated in terms of radiochemical purity by ITLC and HPLC. The content of the vial with maximum radioactivity was diluted to a 5% solution by adding normal saline, followed by passing through a 0.22 mm filter; also, pH was adjusted to 5.5-7.

1.3. Biodistribution of ^{67}Ga-AATS in wild-type rats

For this purpose, 35 µCi of ^{67}Ga-AATS, measured by counting the activity-included syringe before and after the injection in a dose calibrator (with fixed geometry), was injected in each rat. The animals were sacrificed by CO_2 asphyxiation at selected time intervals after injection (0.5, 1, 2, 24, and 48 hours). The tissues (blood, heart, lung, brain, intestine, faeces, skin, stomach, kidney, liver, muscle and bone tissues) were weighed and rinsed with normal saline. The specific activities of the tissues were determined as the percentage of the injected dose per gram of tissue, using a high-purity germanium (HPGe) detector, equipped with a sample-holder device.

1.4. Dosimetric studies

The absorbed dose by each human organ was calculated by RADAR method, based on biodistribution data in rats. The accumulated activity in animals was extrapolated to the accumulated activity in humans, using the method proposed by Sparks and colleagues (equation 1) [14]:

$$\tilde{A}_{\text{human organ}} = \tilde{A}_{\text{animal organ}} \frac{\text{OrganMass}_{\text{human}} / \text{BodyMass}_{\text{human}}}{\text{OrganMass}_{\text{animal}} / \text{BodyMass}_{\text{animal}}} \quad (1)$$

where $\tilde{A}$ is the accumulated activity in the source organ, which can be calculated by equation 2:

$$\tilde{A} = \int_{t_1}^{\infty} A(t)\, dt \quad (2)$$

It should be noticed that A(t) is the activity of each organ at time (t).

The accumulated source activity in each animal organ was calculated by plotting the percentage of the injected dose versus the time and computing the area under the curves. For this purpose, data points, which represent the percentage of the injected dose, were formed. The researchers used a linear approximation between the two experimental points of time.

The curves were extrapolated to infinity by fitting the tail of each curve to a mono-exponential curve with an exponential coefficient equal to the physical decay constant of ^{67}Ga. Afterwards, the area under the curve was calculated. In order to extrapolate the accumulated activity in humans, the mean weight of each organ in a normal human was calculated (Table 1) [12].

Table 1. The mean weight of each organ in an individual with standard weight

Organ	Weight (g)
Bone	5500
Heart	330
Stomach	150
Kidneys	310
Small intestine	650
Spleen	150
Muscle	29000
Liver	1800
Lung	500
Brain	1420
Whole body	73000

The radiation absorbed dose was calculated by RADAR formula (12):

$$D = N \times DF \quad (3)$$

where N is the number of disintegrations occurring in a source organ and DF is:

$$DF = \frac{k \sum_i n_i E_i \phi_i}{m} \quad (4)$$

DF represents the physical decay characteristics of the radionuclide, the range of emitted radiations and the organ size and configuration, expressed in mGy/MBq.s [15]. It should be mentioned that the DFs were obtained, using OLINDA/EXM software (Vanderbilt University, USA) [16].

3. Results

1.1. Production and quality control of $^{67}GaCl_3$

Gallium-67 was prepared as $^{67}GaCl_3$ by proton bombardment (24 MeV) of ^{68}Zn target at a current intensity of 170 mA and charge of 1400 mAh. The chemical separation process was based on a no-carrier-added method. Radiochemical separation was performed by two-step ion exchange chromatography, with a yield higher than 95%. Radionuclidic purity was evaluated by HPGe detector (higher than 99%).

1.2. Preparation and quality control of ^{67}Ga-AATS

The radiolabled ^{67}Ga complex was prepared with high radiochemical purity (>95%, ITLC, >93%, HPLC). ITLC chromatograms of $^{67}GaCl_3$ and ^{67}Ga-AATS solution in 10% ammonium acetate:methanol (1:1) are presented in Figure 1. Also, HPLC chromatogram of ^{67}Ga-AATS is presented in Figure 2.

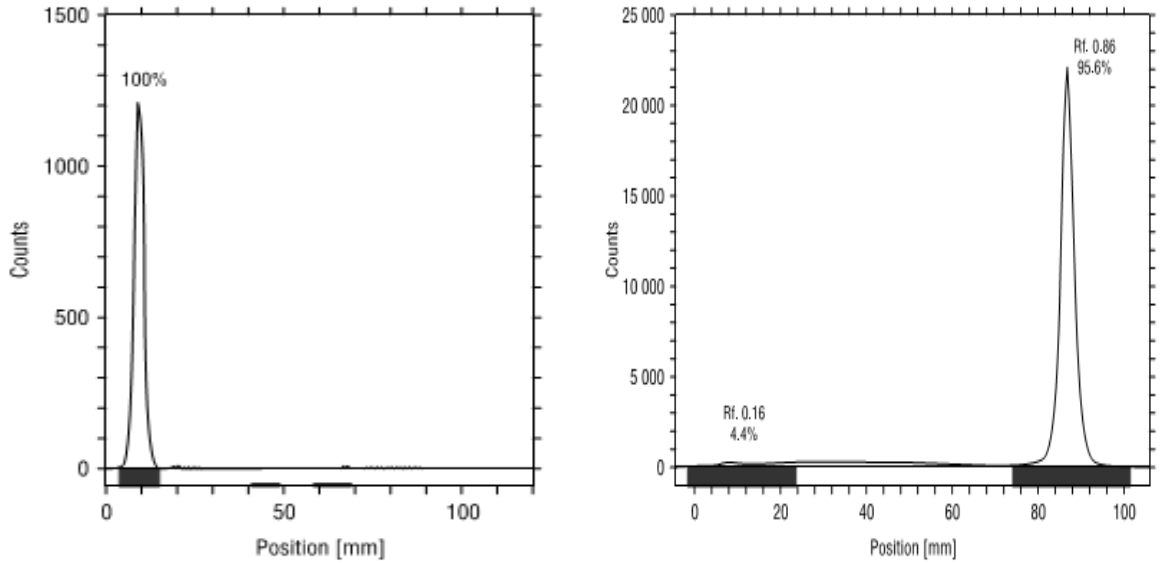

Figure 1. ITLC of [^{67}Ga]$GaCl_3$ (left) and [^{67}Ga]-ATSS (right) on Whatman No. 2 paper, using 10% ammonium acetate:methanol (1:1)

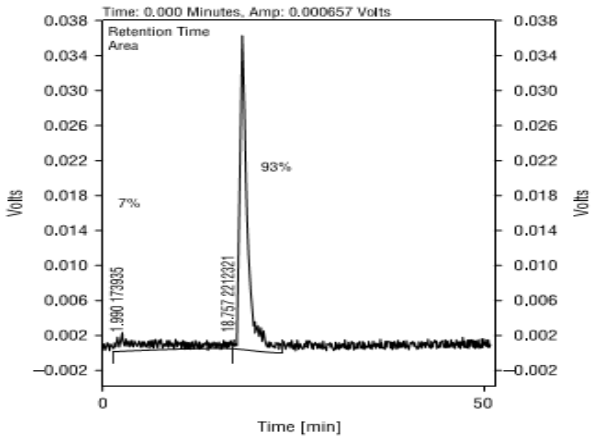

Figure 2: HPLC chromatograms of [^{67}Ga]AATS solution on a reversed-phase column, using acetonitrile + 0.1% TFA/water + 0.1% TFA, 90:10

1.3. Biodistribution of ^{67}Ga-AATS in wild-type rats

The animals were sacrificed by CO_2 asphyxiation at selected time intervals after injection (0.5, 1, 2, 24, and 48h). Dissection started by drawing blood from the aorta, followed by removing the heart, spleen, muscle, bone, kidney, liver, intestine, stomach, lung and skin samples. The tissue uptake was calculated as the percentage of the area under the curve (of the related photo peak) per gram of the tissue (%ID/g).

1.4. Dosimetric studies

Dosimetric evaluation in human organs was performed by RADAR method, based on biodistribution data in rat organs. The clearance curves of each organ of the rats are shown in Figure 3. Also, the absorbed dose in each human organ after ^{67}Ga-AATS injection is presented in Table 2. Since ^{67}Ga-citrate is a common Ga-67 imaging agent, the calculated dose received by various human organs after ^{67}Ga-citrate injection - is provided in this table, as well [17].

Table 2. The absorbed dose by each human organ after the injection of ^{67}Ga-AATS and ^{67}Ga-citrate

Tissues	Absorbed dose (mGy/MBq) ^{67}Ga-AATS	Absorbed dose (mGy/MBq) ^{67}Ga-citrate	Tissue	Absorbed dose (mGy/MBq) ^{67}Ga-AATS	Absorbed dose (mGy/MBq) ^{67}Ga-citrate
Adrenals	0.167	0.13	Muscles	0.081	0.060
Brain	0.073	0.057	Ovaries	0.043	0.082
Breasts	0.039	0.047	Skin	0.067	0.045
Gallbladder wall	0.110	0.082	Pancreas	0.062	0.081
Small intestine	0.073	0.069	Bone surfaces	0.401	0.63
Stomach	0.089	0.059	Spleen	0.117	0.14
Red marrow	0.170	0.21	Testes	0.092	0.056
Cardiac wall	0.054	0.069	Thymus	0.037	0.061
Kidneys	0.112	0.12	Thyroid	0.058	0.062
Liver	0.125	0.12	Uterus	0.086	0.076
Lungs	0.058	0.063	Total body	0.092	0.10
Reference	The present study	[16]	Reference	The present study	[16]

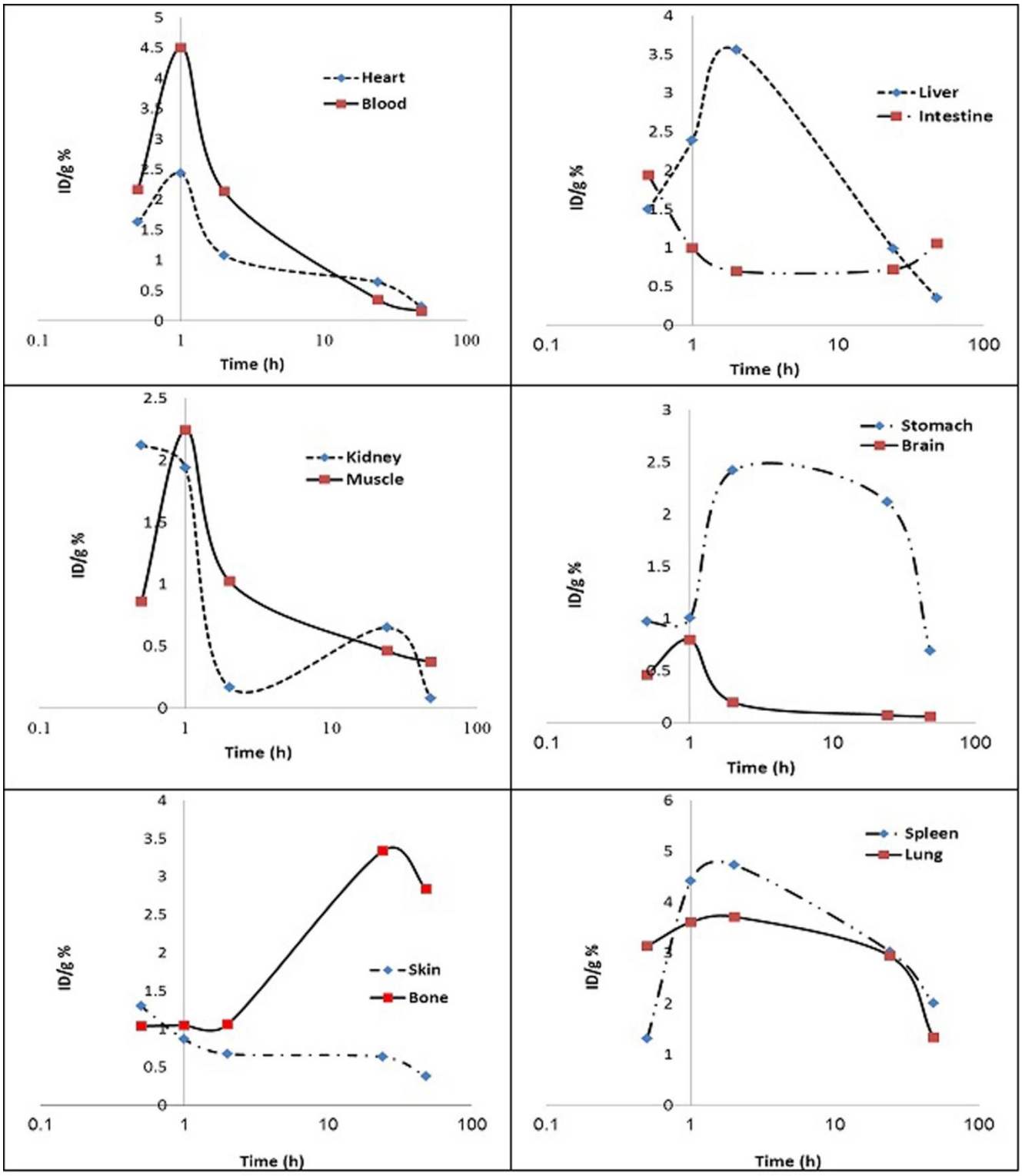

Figure 3. The clearance curves of each organ after ^{67}Ga-AATS injection in rats

4. Discussion

In this study, ^{67}Ga was produced with a radionuclidic purity of higher than 99%. Total labeling procedure for ^{67}Ga -AATS was performed for only 40 minutes and the radiochemical purity of the complex was higher than 93%. The absorbed dose of ^{67}Ga -AATS has not been reported, so far, despite the use of this complex and the direct relationship between the absorbed dose and the effect of radiopharmaceuticals on disease management.

In this study, the absorbed dose of ^{67}Ga-AATS in each human organ was evaluated for the first time and was compared with ^{67}Ga-citrate as the most commonly used form of ^{67}Ga in nuclear medicine. The dosimetric evaluation of the complex, which was performed based on biodistribution data in Wistar rats, demonstrated the highest absorbed dose in bone surfaces (0.401 mGy/MBq). Also, the maximum absorbed dose for ^{67}Ga-citrate was obtained in bone surfaces (0.63 mGy/MBq).

Other organs with considerable absorbed doses included the red marrow, kidneys, liver and spleen with 0.170, 0.112, 0.125 and 0.117 mGy/MBq, respectively. The whole-body absorbed dose after the injection of ^{67}Ga-AATS was 0.092 mGy/MBq, which is comparable to 0.10 mGy/MBq for ^{67}Ga-citrate.

As presented in Table 2, the absorbed dose of each human organ after the injection of ^{67}Ga -AATS was lower than the maximum absorbed dose, proposed by Food and Drug Administration (FDA) [18] and was comparable with the absorbed dose, received by each organ after the injection of ^{67}Ga-citrate as a common radiopharmaceutical. Considering this interesting finding and the significant tumor uptake, which has been demonstrated in previous literature, it seems that ^{67}Ga -AATS can be used as an appropriate SPECT tracer.

5. Conclusion

Calculation of the absorbed dose of the tracer showed that human organs absorbed dose was comparable with the absorbed dose received by each organ after ^{67}Ga-citrate injection. Considering this interesting finding and the significant tumor uptake of the radiolabelled compound, it seems that ^{67}Ga-AATS can be used as an appropriate SPECT tracer.

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